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(54) Title: A METHOD FOR PREDICTING PROTEIN STRUCTURE

(57) Abstract

The present invention relates to methods of predicting the tendency of a portion of a protein to form amphiphilic α or β structure. It is based, at least in part, on the discovery that a number of porin proteins, which were previously believed to contain predominantly α amphiphilic structures, unexpectedly contained substantial amounts of β structure.

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O-sulfate group on residue 2 and the 3-O-sulfate group on residue 4 function in a thermodynamically linked fashion to supply half of the binding energy for interaction with AT, and trigger a conformational event that accelerates neutralization of specific coagulation proteases (11, 12). The amino and ester sulfate groups at residues 5 and 6, as well as carboxyl groups at other sites, provide the other half of the binding energy for interaction with protease inhibitor (10, 11). Furthermore, monosaccharide sequences outside the primary AT binding domain are essential in facilitating inhibition of coagulation proteases other than factor Xa (18, 19).

During the past eight years, several biosynthetic enzymes that generate HSPG^{act} and HSPG^{inact} have been purified. These proteins include an N-acetylglucosamine/glucuronic acid copolymerase (20), N-deacetylase/N-sulfotransferases (NST-1 and NST-2) (21, 22), a glucuronic acid/iduronic acid epimerase (23), an iduronic acid/glucuronic acid 2-O-sulfotransferase (2-OST) (24), a glucosamine 6-O-sulfotransferase (6-OST) (25) and a glucosamine 3-O-sulfotransferase (3-OST) (26, 35). However, the only enzymes that have also been molecularly cloned are two structurally and functionally distinct isoforms of N-deacetylase/N-sulfotransferase (NST-1 from liver and NST-2 from mastocytoma) (27-31), and the 2-OST and epimerase. The above enzymes must function in a coordinated manner to produce the AT binding domain because the abundance of this sequence is much greater than predicted from a random assembly of constituents (32). The postulated regulatory mechanism must direct the biosynthetic enzymes to carry out the appropriate sequence of epimerization/sulfation reactions to generate the AT binding domain (33, 34).

Summary of the Invention

The present invention depends, in part, upon the identification and molecular cloning of novel genes encoding mammalian heparan sulfate D-glucosaminyl 3-O-sulfotransferases (3-OSTs). In particular, as disclosed herein, the present invention provides nucleic acid (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequences for murine 3-OST-1; nucleic acid (SEQ ID NO: 3) and amino acid (SEQ ID NO: 4) sequences for human 3-OST-1; nucleic acid (SEQ ID NO: 5) and amino acid (SEQ ID NO: 6) sequences for human 3-OST-2; nucleic acid (SEQ ID NO: 7) and amino acid (SEQ ID NO: 8) sequences for human 3-OST-3A; nucleic acid (SEQ ID NO: 9) and amino acid (SEQ ID NO: 10) sequences for human 3-OST-3B; and nucleic acid (SEQ ID NO: 11) and amino acid (SEQ ID NO: 12) sequences for human 3-OST-4. In addition, the

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invention provides amino acid (SEQ ID NO: 15) sequences for a *C. elegans* homologue, ce3-OST.

Thus, in one aspect, the present invention provides isolated nucleic acids encoding at least a functional fragment of a 3-OST protein. In preferred embodiments, the nucleic acid encodes a

5   3-OST protein comprising a mature murine or human 3-OST-1. In other embodiments, the nucleic acid encodes a 3-OST protein selected from 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST. In other preferred embodiments, the nucleic acid encodes a 3-O-sulfotransferase domain of a 3-OST protein selected from 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST. In particular embodiments, the nucleic acid comprises a

10   nucleotide sequence selected from nucleotide sequences within: (a) SEQ ID NO: 1; (b) SEQ ID NO: 3; (c) SEQ ID NO: 5; (d) SEQ ID NO: 7; (e) SEQ ID NO: 9; (f) SEQ ID NO: 11; (g) a sequence having at least 60% nucleotide sequence identity with at least one of (a)-(f) and encoding a functional fragment having sequence-specific HS binding affinity or 3-O-sulfotransferase activity; and (h) a sequence differing from a sequence of (a)-(g) only by the

15   substitution of synonymous codons. In other particular embodiments, the present invention provides an isolated nucleic acid encoding a polypeptide selected from: (a) residues 21-52, 260-269, 250-276, 53-311, or 21-307 of SEQ ID NO: 2; (b) residues 21-48, 256-265, 246-272, 49-307, or 21-303 of SEQ ID NO: 4; (c) residues 42-109, 313-325, 303-332, or 110-367 of SEQ ID NO: 6; (d) residues 44-147, 351-363, 341-370, or 148-406 of SEQ ID NO: 8; (e) residues

20   66-132, 336-348, 326-355, or 133-390 of SEQ ID NO: 10; (f) residues 396-408, 386-415, or 207-456 of SEQ ID NO: 12; (g) residues 240-250, 230-257, 23-291 of SEQ ID NO: 15, (h) a sequence having at least 60% amino acid sequence similarity with at least one of (a)-(g) and encoding a functional fragment having sequence-specific HS binding affinity or 3-O-sulfotransferase activity; and (i) a sequence comprising a chimera of at least two of sequences

25   (a)-(h).

In another aspect, the present invention provides isolated nucleic acids comprising at least 16 consecutive nucleotides of a nucleotide sequence selected from SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11.

In another aspect, the present invention provides for cells and cell lines transformed with

30   the nucleic acids of the present invention. Thus, the invention provides host cells transformed with any of the above-described nucleic acids. The transformed host cells may be bacterial, yeast, or insect cells. Preferably, however, the host cells are mammalian cells, including endothelial

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cells, mast cells, fibroblasts, hybridomas, oocytes, and embryonic stem cells. Examples of preferred mammalian cells include COS-7 cells, murine primary cardiac microvascular endothelial cells (CME), murine mast cell line C57.1, primary human endothelial cells of umbilical vein (HUVEC), F9 embryonal carcinoma cells, rat fat pad endothelial cells (RFPEC), L cells (e.g.,

5   murine LTA *tk⁻* cells), and cells derived from the transgenic animals of the invention. The transformed host cells may also be fetal cells, embryonic stem cells, zygotes, gametes, or germ line cells. Transformed embryonic stem cells, zygotes, gametes, and germ line cells, as well as other mammalian cells, may be used to produce transgenic animals in which the expression of 3-OST genes have been altered (e.g., knock-outs, enhanced expression, ectopic expression).

10   In another aspect, the present invention provides substantially pure protein preparations comprising at least a functional fragment of a 3-OST protein. Thus, in one embodiment, the present invention provides a substantially pure protein preparation comprising mature murine 3-OST-1 or mature human 3-OST-1. In another embodiment, the 3-OST protein is selected from the group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST. In

15   another embodiment, the fragment comprises a 3-O-sulfotransferase domain of a 3-OST protein selected from the group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST. In particular embodiments, the present invention provides a substantially pure protein preparation in which the 3-OST protein comprises an amino acid sequence selected from: (a) SEQ ID NO: 2; (b) SEQ ID NO: 4; (c) SEQ ID NO: 6; (d) SEQ ID NO: 8; (e) SEQ ID NO:

20   10; (f) SEQ ID NO: 12; (g) SEQ ID NO 15; and (h) a sequence having at least 60% amino acid similarity with at least one of (a)-(g) and having sequence-specific HS binding affinity or 3-O-sulfotransferase activity. In other particular embodiments, the present invention provides a substantially pure protein preparation in which the 3-OST protein comprises an amino acid sequence selected from: (a) residues 21-52, 260-269, 250-276, 53-311, or 21-307 of SEQ ID

25   NO: 2; (b) residues 21-48, 256-265, 246-272, 49-307, or 21-303 of SEQ ID NO: 4; (c) residues 42-109, 313-325, 303-332, or 110-367 of SEQ ID NO: 6; (d) residues 44-147, 351-363, 341-370, or 148-406 of SEQ ID NO: 8; (e) residues 66-132, 336-348, 326-355, or 133-390 of SEQ ID NO: 10; (f) residues 396-408, 386-415, or 207-456 of SEQ ID NO: 12; (g) residues 240-250, 230-257, 23-291 of SEQ ID NO: 15; (h) a sequence having at least 60%

30   amino acid sequence similarity with at least one of (a)-(g) and encoding a functional fragment having sequence-specific HS binding affinity or 3-O-sulfotransferase activity; and (i) a sequence comprising a chimera of at least two of sequences (a)-(h).

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In another aspect, the present invention provides for antibodies and methods for making antibodies which selectively bind with the 3-OST proteins. These antibodies include monoclonal and polyclonal antibodies, as well as functional antibody fragments such as F(ab) and Fc.

In another aspect, the present invention provides for methods for producing the above-described proteins. Thus, in one set of embodiments, the isolated nucleic acids of the invention may be used to transform host cells or create transgenic animals which express the proteins of the invention. The proteins may then be substantially purified from the cells or animals by standard methods. Alternatively, the isolated nucleic acids of the invention may be used in cell-free *in vitro* translation systems to produce the proteins of the invention.

In another aspect, the present invention provides methods for 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan or proteoglycan polysaccharides by contacting the preparation with at least a 3-O-sulfotransferase domain of a 3-OST protein in the presence of a sulfate donor under conditions which permit sulfation of the residues, and wherein the 3-OST protein is selected from 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST proteins, as well as conservative substitution variants and/or chimeras thereof. In particular embodiments, the present invention provides methods for 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan or proteoglycan polysaccharides in which the polysaccharides include a polysaccharide sequence of GlcA→GlcNS ±6S. These methods comprise contacting the GlcA→GlcNS ±6S-containing polysaccharide preparation with a 3-OST-1 protein in the presence of a sulfate donor under conditions which permit the 3-OST-1 to convert the GlcA→GlcNS ±6S sequence to GlcA→GlcNS 3S ±6S. In particular embodiments, the GlcA→GlcNS ±6S sequence comprises a part of an HS^{act} precursor sequence (i.e., IdoA→ GlcNAc 6S→GlcA→GlcNS ±6S→IdoA 2S→GlcNS 6S or IdoA→GlcNS 6S→GlcA→GlcNS ±6S→IdoA 2S→GlcNS 6S) or a part of an HS^{inact} precursor sequence (i.e., IdoA→GlcNAc→ GlcA→GlcNS ±6S→IdoA 2S→GlcNS 6S; IdoA→GlcNS→GlcA→GlcNS ±6S→IdoA 2S→GlcNS 6S; IdoA→GlcNAc 6S→GlcA→GlcNS ±6S→IdoA 2S→GlcNS; or IdoA→ GlcNS 6S→GlcA→GlcNS ±6S→IdoA 2S→GlcNS). Conversion of the HS^{act} precursor pool to HS^{act} increases the fraction with AT-binding activity and is particularly useful in the production of anticoagulant heparan sulfate products. Thus, in another embodiment, the present invention provides for means of enriching the AT-binding fraction of a heparan sulfate pool by contacting the polysaccharide preparation with 3-OST-1 protein in the presence of a sulfate donor under

conditions which permit the 3-OST HS^{act} conversion activity. The 3-OST-1 protein for use in these methods is selected from murine 3-OST-1, human 3-OST-1, mature murine 3-OST-1, mature human 3-OST-1, a functional fragment of a 3-OST-1 having 3-O-sulfotransferase activity, a conservative substitution variant of 3-OST-1 having 3-O-sulfotransferase activity, and a

5   chimeric 3-OST-1 having 3-O-sulfotransferase activity. In preferred embodiments, the sulfate donor is 3'-phospho-adenosine 5'-phosphosulfate (PAPS).

Similarly, the present invention provides methods for 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan or proteoglycan polysaccharides by contacting the preparation with at least a 3-O-sulfotransferase domain of a 3-OST protein in the presence of a

10   sulfate donor under conditions which permit sulfation of the residues, and wherein the 3-OST protein is selected from 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, ce3-OST and conservative substitution variants or chimeras thereof. In particular embodiments, the present invention provides methods for 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan or proteoglycan polysaccharides in which the polysaccharides include a polysaccharide sequence

15   of GlcA 2S→GlcNS. These methods comprise contacting the GlcA 2S→GlcNS-containing polysaccharide preparation with a 3-OST-2 protein in the presence of a sulfate donor under conditions which permit the 3-OST-2 protein to convert the GlcA 2S→GlcNS sequence to GlcA 2S→GlcNS 3S. In particular embodiments, the GlcA 2S→GlcNS sequence comprises a part of a GlcNS→GlcA 2S→GlcNS sequence. In other particular embodiments, the present invention

20   provides methods for 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan or proteoglycan polysaccharides in which the polysaccharides include a polysaccharide sequence of IdoA 2S→GlcNS. These methods comprise contacting the IdoA 2S→GlcNS-containing polysaccharide preparation with a 3-OST-3 protein in the presence of a sulfate donor under conditions which permit the 3-OST-3 protein to convert the IdoA 2S →GlcNS sequence to IdoA

25   2S→GlcNS 3S. In particular embodiments, the IdoA 2S→GlcNS sequence comprises a part of a GlcNS→IdoA 2S→GlcNS sequence. The 3-OST proteins for use in these methods are selected from 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, ce3-OST, functional fragments of these 3-OSTs having 3-O-sulfotransferase activity, conservative substitution variants of these 3-OSTs having 3-O-sulfotransferase activity, and chimeric 3-OSTs having 3-O-sulfotransferase activity. In

30   preferred embodiments, the sulfate donor is 3'-phospho-adenosine 5'-phosphosulfate (PAPS).

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In another aspect, the present invention provides methods for partially sequencing complex polysaccharides such as heparan sulfates or other glycosaminoglycans (GAGs). In these methods, a pool of polysaccharides which includes sequences which may be 3-O-sulfated is contacted with a 3-OST protein in the presence of a sulfate donor (e.g., PAPS) under conditions

5   which permit sulfation by the 3-OST. The treated polysaccharides are then subjected to degradation by enzymes which degrade polysaccharides in a sequence-specific manner (e.g., polysaccharide lyases; heparinase I, II or III; heparitinase) and the size profile of the resulting fragments is determined. An identical pool which has not been treated with 3-OST is similarly cleaved by the same enzymes and a size profile determined. Changes in the size profiles indicate

10   that 3-OST activity has modified the saccharide units so as to prevent (or permit) cleavage at sites which previously were (or were not) cleaved. Thus, comparison of the profiles will indicate positions at which the target sequences for 3-OST activity are present and provide a partial polysaccharide sequence.

In another embodiment, the sequence of complex polysaccharides such as HS or GAGs

15   may be partially determined using sequence specific polysaccharide affinity fractionation. To this end, 3-OST proteins which lack enzymatic function but retain sequence-specific HS or GAG binding capacity can be identified or produced (e.g., altering or deleting a portion of the catalytic ST domain by site-directed mutagenesis or deletion mutagenesis). These inactive forms will bind HS or GAGs in a sequence dependent manner and allow sequence-specific saccharide affinity

20   fractionation from complex mixtures of GAGs. The purified structures may be degraded in a step-wise fashion with exolytic, endolytic enzymes and/or nitrous acid, and the resulting degradation products can be compared to standard compounds of known structure. This method will allow the quantitation and characterization of known structures contained within unknown complex polysaccharide samples.

25   In another embodiment, partial sequence information can be obtained using the 3-OSTs of the invention or other heparan sulfate sequence specific binding ligands as protective groups prior to treating the HS or GAG with modifying agents that detectably alter the HS or GAG. Useful protective groups include catalytically inactive enzymes, chimeric enzymes and small molecule ligands with identified sequence binding specificities. The protecting group is contacted with the

30   heparan or other glycosaminoglycans (GAGs), and the resultant complex is treated with one or more modifying agents. Useful modifying agents include catalytically active heparan lyases, sulfotransferases, N-deacetylases, N-acetyltransferases, epimerases, or chimeric proteins of the

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invention. In embodiments where multiple protecting groups and/or modifying reagents are used in combination, the sample is first contacted with the protective group, then one or more modifying reagents may be with contacted with the protected polysaccharide, either simultaneously or in turn. The protective group(s) will interfere with the ability of a modifying

5   agent to interact with, attach to and/or cleave specific GAG sequence motifs. The sample can then be analyzed for ligand-specific protection and/or cleavage to elucidate the sequence of the original GAG using separation and/or quantitation using methods known in the art.

In another set of embodiments, the present invention provides isolated nucleic acids comprising a genetic regulatory sequences of a 3-OST gene operably joined to a marker gene.

10   Such regulatory sequences include 5' untranslated regions such as promoter and operator sequences. The 5' regulatory sequences of the human 3-OST-4 gene (as well as coding regions) are disclosed herein as SEQ ID NO: 16. Such regulatory regions may be used to transform host cells, which are useful in methods of identifying compounds capable of modulating the expression of the 3-OST gene. Thus, in such methods, a candidate compound is contacted with a host cell

15   transformed with a marker gene operably joined to the 3-OST regulatory regions, and changes in expression of the marker gene are indicative of the ability of the candidate compound to modulate 3-OST expression.

In another aspect, the present invention also provides methods for diagnosing individuals with disorders involving heparan sulfate biosynthesis comprising assaying such individuals for the

20   presence of mutations in 3-OST genes/proteins. Such assays include nucleic acid based assays (employing the nucleic acids of the present invention), protein based assays (employing the antibodies of the present invention), and HS based assays employing the glycosaminoglycan sequencing methods of the present invention.

These and other aspects of the present invention will be apparent to one of ordinary skill

25   in the art from the following detailed description.

Brief Description of the Drawings

Fig. 1 is an alignment of the amino acid sequences of murine and human 3-OST-1 proteins showing the high degree of homology. Vertical bars (|) between residues indicate identical residues.

30   Fig. 2 is an alignment of the sulfotransferase domains of human NST-1, human NST-2, *C. elegans* 3-OST, human 3-OST-4, human 3-OST-3A, human 3-OST-2, and human 3-OST-1.

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Fig. 3 is a schematic depiction of the structures of the 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B and 3-OST-4 proteins.

Detailed Description of the Invention

Definitions

5   In order to more clearly and distinctly point out and describe the subject matter that applicants regard as the invention, the following definitions are provided for certain terms used in the following written description and the appended claims.

Isolated nucleic acids. As used herein with respect to nucleic acids derived from naturally-occurring sequences, the term "isolated nucleic acid" means a ribonucleic or deoxyribonucleic
10   acid which comprises a naturally-occurring nucleotide sequence and which is manipulable by standard recombinant DNA techniques, but which is not covalently joined to the nucleotide sequences that are immediately contiguous on its 5' and 3' ends in the naturally-occurring genome of the organism from which it is derived. As used herein with respect to synthetic nucleic acids, the term "isolated nucleic acid" means a ribonucleic or deoxyribonucleic acid which comprises a
15   nucleotide sequence which does not occur in nature and which is manipulable by standard recombinant DNA techniques. An isolated nucleic acid is manipulable by standard recombinant DNA techniques when it may be used in, for example, amplification by polymerase chain reaction (PCR), *in vitro* translation, ligation to other nucleic acids (e.g., cloning or expression vectors), restriction from other nucleic acids (e.g., cloning or expression vectors), transformation of cells,
20   hybridization screening assays, or the like. The term "isolated nucleic acids" is also intended to embrace synthetic oligonucleotides such as peptide nucleic acids (PNAs), nucleotides joined by phosphorothioate or other non-phosphodiester linkages, nucleic acids incorporating functionally equivalent nucleotide analogs, and the like.

Transformation As used herein, means any method of introducing exogenous a nucleic
25   acid into a cell including, but not limited to, transformation, transfection, electroporation, microinjection, direct injection of naked nucleic acid, particle-mediated delivery, viral-mediated transduction or any other means of delivering a nucleic acid into a host cell which results in transient or stable expression of said nucleic acid or integration of said nucleic acid into the genome of said host cell or descendant thereof.

30   Substantially pure. As used herein with respect to protein preparations, the term "substantially pure" means a preparation which contains at least 60% (by dry weight) the protein of interest, exclusive of the weight of other intentionally included compounds. Preferably the

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preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by dry weight the protein of interest, exclusive of the weight of other intentionally included compounds. Purity can be measured by any appropriate method, e.g., column chromatography, gel electrophoresis, or HPLC analysis. If a preparation intentionally includes two or more

5   different proteins of the invention, a "substantially pure" preparation means a preparation in which the total dry weight of the proteins of the invention is at least 60% of the total dry weight, exclusive of the weight of other intentionally included compounds. Preferably, for such preparations containing two or more proteins of the invention, the total weight of the proteins of the invention be at least 75%, more preferably at least 90%, and most preferably at least 99%, of

10   the total dry weight of the preparation, exclusive of the weight of other intentionally included compounds. Thus, if the proteins of the invention are mixed with one or more other proteins (e.g., serum albumin, 6-OST) or compounds (e.g., diluents, detergents, excipients, salts, polysaccharides, sugars, lipids) for purposes of administration, stability, storage, and the like, the weight of such other proteins or compounds is ignored in the calculation of the purity of the

15   preparation.

Similarity. As used herein with respect to amino acid sequences, the "similarity" between two sequences means the percentage of amino acid residue positions, after aligning the sequences according to standard techniques, at which the two sequences have identical or similar residues. In general, "similar" residues include those which are regarded in the art as "conservative

20   substitutions" (see, e.g., Dayhoff et al. (1978), Atlas of Protein Sequence and Structure Vol. 5 (Suppl. 3), pp. 354-352, Natl. Biomed. Res. Found., Washington, D.C.); which fall within the groups (a) methionine, leucine, isoleucine and valine, (b) phenylalanine, tyrosine and tryptophan, (c) lysine, arginine and histidine, (d) alanine and glycine, (e) serine and threonine, (f) glutamine and asparagine, and (g) glutamate and aspartate; or which are otherwise shown to have no

25   substantial effect on the biological activity of the protein. Numerical values for similarity were determined using the PileUp program. This program performed multiple sequence alignments based on methods of Feng and Doolittle (1987) J. Mol. Evol. 35: 351-360, and Higgins and Sharp (1998), CABIOS 5:151-153. Using these methods for each sequence alignment, the gap weight was set at 3.0 and the gap length was set at 0.10. Percentages of similarity recited in the

30   appended claims may be determined by these methods.

Chimeric protein. As used herein, the term "chimeric protein" means a protein having an amino acid sequence which is a positionally conserved combination of the amino acid sequences

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of two or more other proteins. Thus, for a chimera of two or more reference proteins, the amino acid sequences of the reference proteins are aligned by standard techniques to identify residues which correspond at each position, allowing for relative insertions/deletions as necessary. Then, for each amino acid position of the chimeric protein, an amino acid residue is selected from the

5   residues present at corresponding positions in the two or more reference proteins (allowing for no residue in the chimera when deletions are present amongst the reference proteins). The resultant chimera has an amino acid sequence which is a combination of the reference amino acid sequences, in which the relative position of each residue selected from the reference sequences is conserved within the chimera.

10   Heparan sulfate. As used herein, the term "heparan sulfate" or the abbreviation "HS" means a polysaccharide of the form ([→4-D-GlcApβ1 or →4-L-IdoApα1] →4-D-GlcNp[Ac or S]α1→)n which is modified to a variable extent by sulfation of the 2-O-position of Glc and Ido residues, and the 6-O- and 3-O- positions of GlcN[Ac or S] residues. Therefore, this definition encompasses all glycosaminoglycan compounds referred to as heparan(s), heparan sulfate(s),

15   heparin(s), heparin sulfate(s), heparitin(s), heparitin sulfate(s), heparanoid(s), heparosan(s). The heparan molecules may be pure glycosaminoglycans or can be linked to other molecules including other polymers such as proteins, and lipids, or small molecules such as biotin.

The Heparan Sulfate D-Glucosaminyl 3-O-Sulfotransferases The present invention depends, in

20   part, upon the identification and molecular cloning of cDNAs encoding mammalian heparan sulfate D-glucosaminyl 3-O-sulfotransferases (3-OSTs). These proteins have been designated 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, and 3-OST-4. In addition, a nematode 3-OST from *C. elegans*, ce3-OST, has been identified.

        3-OST-1s. Disclosed herein are the isolation and identification of murine and human 3-

25   OST-1 cDNAs (SEQ ID NO: 1 and SEQ ID NO: 3, respectively). The coding regions of these cDNAs extend from, respectively, nucleotide positions 323-1255 of SEQ ID NO: 1 and positions 119-1039 of SEQ ID NO : 3. The protein coding portions of the cDNAs are 85% identical and encode proteins of 311 and 307 amino acids (SEQ ID NO: 2 and SEQ ID NO: 4, respectively) which are 93% similar. The murine and human protein sequences are aligned in Figure 1. Each

30   protein includes a twenty residue presumptive signal peptide (residues 1-20 of SEQ ID NO: 2 and SEQ ID NO: 4) which is cleaved off to form the mature form of these proteins. The mouse 3-

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OST-1 contains an extra four residues (Ala^{24}-Pro^{25}-Gly^{26}-Pro^{27}) not found in the human form.

Each protein has five potential N-glycosylation sites (at residues 52-54, 141-143, 196-198, 246-248 and 253-255 of SEQ ID NO: 2, and residues 48-50, 137-139, 192-194, 242-244, 249-251 of SEQ ID NO: 4). N-glycosylation of at least some of these sites appears important to 3-OST

5   protein stability, specificity and/or activity. After the 3-OST-1 signal peptide, there is a domain rich in the residues S, P, L, A, and G (SPLAG-rich domain) (residues 21-52 of SEQ ID NO: 2 and residues 21-48 of SEQ ID NO: 4). 3-OST-1 and all known NST species possess a homologous carboxy terminal sulfotransferase (ST) domain of ~260 amino acids (residues 53-311 of SEQ ID NO: 2 and residues 49-307 of SEQ ID NO: 4) that exhibits homology to all known

10   sulfotransferases and which includes the minimal fragment necessary for sulfation activity. Figure 2 shows a sequence alignment of the ST domains of the sulfotransferases NST-1 (SEQ ID NO: 13), NST-2 (SEQ ID NO: 14), OST-1, OST-2, OST-3A/B, and OST-4. Within this region is a conserved sequence (at residues 260-269 of SEQ ID NO: 2, and 256-265 of SEQ ID NO: 4) which is a presumptive cysteine-bridged peptide loop thought to be involved in heparan sulfate

15   substrate specificity. This cysteine-bridged peptide loop is part of the larger HS-binding domain (residues 250-276 of SEQ ID NO: 2 and 246-272 of SEQ ID NO: 4). A conserved lysine residue (residue 68 of SEQ ID NO: 2, and 64 of SEQ ID NO: 4) is presumptively catalytic.

The 3-OST-1 proteins have 3-O-sulfotransferase activity on polysaccharide sequences including the sequence GlcA→GlcNS ±6S, and convert this polysaccharide sequence to the

20   sequence to GlcA→GlcNS 3S ±6S. Of particular importance, the 3-OST-1 proteins are useful in converting HS^{act} precursor sequences (i.e., IdoA→GlcNAc 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S; or IdoA→GlcNS 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S) to HS^{act}. The 3-OST-1 proteins are highly expressed in endothelial cells, brain and kidney tissues, and to a lesser extent in heart, lung, skeletal muscle and placenta. The human 3-OST-1 gene has been

25   syntactically localized to chromosome 4, and more particularly to chromosome segment 4p15-16.

3-OST-2s. Also disclosed herein are the isolation and identification of a human 3-OST-2 cDNA (SEQ ID NO: 5). The coding region of this cDNA extends from nucleotide positions 73-1173 of SEQ ID NO: 5. The cDNA encodes a protein of 367 amino acids (SEQ ID NO: 6). The protein has four potential N-glycosylation sites (at residues 102-104, 193-195, 235-237 and 306-

30   308 of SEQ ID NO: 6). N-glycosylation of at least some of these sites appears important to 3-OST protein stability, specificity and/or activity. The 3-OST-2 protein has a putative N-terminal

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cytoplasmic domain (residues 1-19 of SEQ ID NO: 6), followed by a putative transmembrane domain (residues 20-41 of SEQ ID NO: 6), followed by a SPLAG-rich domain (residues 42-109 of SEQ ID NO: 6). This is followed by the characteristic carboxy terminal ST domain of ~260 amino acids (residues 110-367 of SEQ ID NO: 6) that exhibits homology to all known sulfotransferases and which includes the minimal fragment necessary for sulfation activity. Within this region is a conserved sequence (at residues 313-325 of SEQ ID NO: 6) which is a presumptive cysteine-bridged peptide loop thought to be involved in heparan sulfate substrate specificity. This cysteine-bridged peptide loop is part of the larger HS-binding domain (residues 303-332 of SEQ ID NO: 6). A conserved lysine residue (residue 24 of SEQ ID NO: 6) is presumptively catalytic. A cDNA of an allelic variant has also been identified, which includes four silent nucleotide substitutions (G→A at bp 804, T→G at bp 1249, T→C at bp 1350, and C→T at bp 1507 of SEQ ID NO: 5) which do not affect the encoded protein.

The 3-OST-2 proteins have 3-O-sulfotransferase activity on polysaccharide sequences including the sequences GlcA 2S→GlcNS or GlcNS→GlcA 2S→GlcNS, and convert these polysaccharide sequences to GlcA 2S→GlcNS 3S or GlcNS→GlcA 2S→GlcNS 3S, respectively. The 3-OST-2 proteins are not expressed in endothelial cells, but are highly expressed in brain tissues, and to a lesser extent in heart, lung, skeletal muscle and placenta. The human 3-OST-2 gene has been localized to chromosome 16, and more particularly to chromosome segment 16p12.3.

3-OST-3As. Also disclosed herein are the isolation and identification of a human 3-OST-3A cDNA (SEQ ID NO: 7). The coding region of this cDNA extends from nucleotide positions 799-2016 of SEQ ID NO: 7. The cDNA encodes a protein of 406 amino acids (SEQ ID NO: 8). The protein has two potential N-glycosylation sites (at residues 273-275 and 344-346 of SEQ ID NO: 8). N-glycosylation of one or more of these sites appears important to 3-OST protein stability, specificity and/or activity. The 3-OST-3A protein has a putative N-terminal cytoplasmic domain (residues 1-24 of SEQ ID NO: 8), followed by a putative transmembrane domain (residues 25-43 of SEQ ID NO: 8), followed by a SPLAG-rich domain (residues 44-147 of SEQ ID NO: 8). This is followed by the characteristic carboxy terminal ST domain of ~260 amino acids (residues 148-406 of SEQ ID NO: 8) that exhibits homology to all known sulfotransferases and which includes the minimal fragment necessary for sulfation activity. Within this region is a conserved sequence (at residues 351-363 of SEQ ID NO: 8) which is a presumptive cysteine-bridged peptide loop thought to be involved in heparan sulfate substrate specificity. This

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cysteine-bridged peptide loop is part of the larger HS-binding domain (residues 341-370 of SEQ ID NO: 8). A conserved lysine residue (residue 162 of SEQ ID NO: 8) is presumptively catalytic.

The 3-OST-3A proteins have 3-O-sulfotransferase activity on polysaccharide sequences including the sequences IdoA 2S→GlcNS or GlcNS→IdoA 2S→GlcNS, and convert these

5   polysaccharide sequences to IdoA 2S→GlcNS 3S or GlcNS→IdoA 2S→GlcNS 3S, respectively. The 3-OST-3A proteins are not expressed in endothelial cells, but are highly expressed in kidney, placenta and liver tissues, and to a lesser extent in brain, heart, lung, and skeletal muscle.

3-OST-3Bs. Also disclosed herein are the isolation and identification of a human 3-OST-3B cDNA (SEQ ID NO: 9). The coding region of this cDNA extends from nucleotide positions

10   331-1500 of SEQ ID NO: 9. The cDNA encodes a protein of 390 amino acids (SEQ ID NO: 10). The protein has two potential *N*-glycosylation sites (at residues 258-260 and 329-331 of SEQ ID NO: 10). *N*-glycosylation of one or more of these sites appears important to 3-OST protein stability, specificity and/or activity. The 3-OST-3B protein has a putative N-terminal cytoplasmic domain (residues 1-32 of SEQ ID NO: 10), followed by a putative transmembrane domain

15   (residues 33-65 of SEQ ID NO: 10), followed by a SPLAG-rich domain (residues 66-132 of SEQ ID NO: 10). This is followed by the characteristic carboxy terminal ST domain of ~260 amino acids (residues 133-390 of SEQ ID NO: 10) that exhibits homology to all known sulfotransferases and which includes the minimal fragment necessary for sulfation activity. Within this region is a conserved sequence (at residues 336-348 of SEQ ID NO: 10) which is a presumptive cysteine-

20   bridged peptide loop thought to be involved in heparan sulfate substrate specificity. This cysteine-bridged peptide loop is part of the larger HS-binding domain (residues 326-355 of SEQ ID NO: 10). A conserved lysine residue (residue 147 of SEQ ID NO: 10) is presumptively catalytic.

The 3-OST-3B proteins have 3-O-sulfotransferase activity on polysaccharide sequences

25   including the sequences IdoA 2S→GlcNS or GlcNS→IdoA 2S→GlcNS, and convert these polysaccharide sequences to IdoA 2S→GlcNS 3S or GlcNS→IdoA 2S→GlcNS 3S, respectively. The 3-OST-3A proteins are not expressed in endothelial cells, but are highly expressed in kidney, placenta and liver tissues, and to a lesser extent in brain, heart, lung, and skeletal muscle.

3-OST-4s. Also disclosed herein are the isolation and identification of a human 3-OST-4

30   nucleic acid sequence (SEQ ID NO: 11). This sequence represents is a possible or predicted heteronuclear RNA species, and is a composite of 5' genomic sequences information and an

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overlapping partial cDNA. The coding region of this sequence extends from nucleotide positions 847-2214 of SEQ ID NO: 11, and encodes a protein of 456 amino acids (SEQ ID NO: 12). The protein has two potential *N*-glycosylation sites (at residues 318-320 and 389-391 of SEQ ID NO: 12). *N*-glycosylation of one or more of these sites appears important to 3-OST protein stability,

5   specificity and/or activity. The 3-OST-4 includes the characteristic carboxy terminal ST domain of ~260 residues (residues 207-456 of SEQ ID NO: 12) that exhibits homology to all known sulfotransferases and which includes the minimal fragment necessary for sulfation activity. Within this region is a conserved sequence (at residues 396-408 of SEQ ID NO: 12) which is a presumptive cysteine-bridged peptide loop thought to be positioned near the active site. This

10   cysteine-bridged peptide loop is part of the larger HS-binding domain (residues 386-415 of SEQ ID NO: 12). A conserved lysine residue (residue 207 of SEQ ID NO: 12) is presumptively catalytic.

       The 3-OST-4 proteins have sulfotransferase activity, but the sequence specificity of this activity has not yet been determined. The 3-OST-4 proteins appear to be expressed at detectable

15   levels only in the brain. The human 3-OST-4 gene has been localized to chromosome 16, and more particularly to chromosome segment 16p11.

       C. elegans 3-OSTs. Also disclosed herein is the identification of a *C. elegans* homologue of the human 3-OSTs, ce3-OST. This protein is disclosed as SEQ ID NO: 15, and includes the characteristic carboxy terminal ST domain of ~260 residues (residues 23-291 of SEQ ID NO: 15)

20   that exhibits homology to all known sulfotransferases and which includes the minimal fragment necessary for sulfation activity. Within this region is a conserved sequence (at residues 240-250 of SEQ ID NO: 15) which is a presumptive cysteine-bridged peptide loop thought to be positioned near the active site. This cysteine-bridged peptide loop is part of the larger HS-binding domain (residues 230-257 of SEQ ID NO: 15). A conserved lysine residue (residue 38 of SEQ

25   ID NO: 15) is presumptively catalytic.

       The *C. elegans* 3-OST proteins have sulfotransferase activity, but the sequence specificity of this activity has not yet been determined. BLAST and Genefinder analysis of genomic cosmids predicts that ce3-OST is an intraluminal resident protein of 291 residues encoded by 4 exons (clone F52B10, GBan U41990; residues 26317-26090, 21886-21732, 21682-21395, and 21345-

30   21140).

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The homology between the sulfotransferase domain of the ce3-OST and the human 3-OST and NST proteins is illustrated in Fig. 2. Based on this sequence alignment, one may also produce chimeric proteins between and the *C elegans* protein and its human homologues.

Isolated Nucleic Acids

5   In one aspect, the present invention provides isolated nucleic acids encoding 3-OST proteins or functional fragments thereof. In preferred embodiments, the 3-OST proteins are 3-OST-1 proteins, 3-OST-2 proteins, 3-OST-3A proteins, 3-OST-3B proteins, 3-OST-4 proteins, or ce3-OST proteins. In particularly preferred embodiments, the 3-OST proteins are those disclosed as SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10,

10   SEQ ID NO: 12, or SEQ ID NO: 15. As shown in the examples below, the isolated nucleic acids encoding all or a portion of one mammalian 3-OST protein may be used to isolate homologues in other species by standard techniques known to those of ordinary skill in the art. Thus, the present invention also enables isolated nucleic acids encoding the 3-OST proteins of other mammalian species including, for example, rats, goats, sheep, cows, pigs, and non-human primates. Similarly,

15   the isolated nucleic acids disclosed herein may be used to screen additional human or other mammalian genetic libraries (e.g., genomic or cDNA libraries) to identify allelic variants of the particularly disclosed sequences. Thus, the present invention also enables isolated nucleic acids encoding human and other mammalian 3-OST allelic variants.

In another aspect, the present invention provides isolated nucleic acids encoding functional

20   fragments of 3-OST proteins, 3-OST protein variants in which conservative substitutions have been made for certain residues, or encoding chimeric 3-OST proteins in which the sequences of two or more 3-OST proteins have been mixed, to produce non-naturally occurring variants which retain sequence-specific HS binding affinity and/or 3-O-sulfotransferase activity. The preferred amino acid sequences of such variants are described below.

25   In preferred embodiments, the isolated nucleic acids encoding a mammalian 3-OST or functional fragment thereof have at least 60%, preferably at least 70%, and more preferably at least 80% nucleotide sequence identity to the coding regions of the mammalian 3-OST sequences particularly disclosed herein (SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and SEQ ID NO: 11), and encode at least a functional fragment having sequence-

30   specific HS binding affinity and/or 3-O-sulfotransferase activity. Most preferably, the sequences have at least 90% or 95% nucleotide sequence identity to the disclosed reference sequences.

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As will be apparent to one of ordinary skill in the art, the degeneracy of the genetic code allows for numerous nucleotide substitutions in a given coding sequence which do not affect the amino acid sequence of the encoded protein. Thus, the present invention also provides for isolated nucleic acids which differ from any of the above-described sequences only by the
5   substitution of such synonymous codons.

The isolated nucleic acids of the present invention may be joined to other nucleic acid sequences for use in various applications. Thus, for example, the isolated nucleic acids of the invention may be ligated into cloning or expression vectors, as are commonly known in the art and as described in the examples below. In addition, the nucleic acids of the invention may be
10   joined in-frame to sequences encoding another polypeptide so as to form a fusion protein, as is commonly known in the art and as described in the examples below. Thus, in certain embodiments, the present invention provides cloning, expression and fusion vectors comprising any of the above-described nucleic acids.

In another aspect, the isolated nucleic acids of the present invention may comprise only a
15   portion of a nucleotide sequence encoding a complete mammalian 3-OST protein. For example, and as described more fully below, the 3-OST-1 proteins comprise a signal sequence which is removed post-translationally to yield the mature proteins. In some instances (e.g., when translating 3-OST-1 proteins *in vitro*), it may be preferable to employ an isolated nucleic acid which encodes only the mature protein. In addition, the four C-terminal residues of 3-OST-1 are
20   believed to be involved in localization of the protein within the Golgi apparatus. In some instances (e.g., when encoding 3-OST-1 proteins for use *in vitro*), it may be preferable to employ an isolated nucleic acid which does not encode these residues, as they will be unnecessary for *in vitro* function. As described above, an approximately 260 residue portion of the 3-OST proteins includes the catalytically active region (ST domain) and, therefore, it may be preferable to employ
25   an isolated nucleic acid which encodes only this functional fragment which retains 3-O-sulfotransferase activity. Thus, in certain preferred embodiments, the present invention provides isolated nucleic acid sequences encoding mature forms of a mammalian 3-OST-1 protein, C-terminally truncated forms of the 3-OST proteins, or minimal functional fragments of the 3-OST proteins. In addition, as described above, these sequences may also encode conservative
30   substitution variants or chimeras of 3-OST proteins, and may include synonymous codon substitutions.

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In another aspect, the present invention provides for nucleic acids which comprise a sequence of at least 16-18, preferably 18-20 consecutive nucleotides from any one of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9 and SEQ ID NO: 11. Such nucleic acid sequences have utility for determining the levels of expression of 3-OST

5   transcripts in cells or tissues, for identifying tissues in which the 3-OST genes are differentially expressed (see above), for encoding peptide fragments which may be used to raise antibodies to corresponding regions of the 3-OST proteins, identifying chromosomes bearing the corresponding 3-OST sequences (see above), for priming polymerase chain reaction amplification of 3-OST sequences (e.g., prior to *in vitro* translation, see below), and for various other utilities which will

10   be apparent to those skilled in the art. Particularly preferred sequences for PCR amplification include those which are 5' to and/or include the initiation codon, which are 5' to and/or include the codons encoding the signal peptide cleavage site, or which are 3' to and/or include the termination codon. Sequences useful for encoding peptide fragments include those which are located within the coding region.

15   Cell Lines and Transgenic Animals

The present invention also provides for cells or cell lines, both prokaryotic and eukaryotic, into which have been introduced the nucleic acids of the present invention so as to cause clonal propagation of those nucleic acids and/or expression of the proteins or peptides encoded thereby. Such cells or cell lines have utility in the propagation and production of the nucleic acids of the

20   invention, as well as the production of the proteins of the present invention. As used herein, the term "transformed cell" is intended to embrace any cell, or the descendant of any cell, into which has been introduced any of the nucleic acids of the invention, whether by transformation, transfection, transduction, infection, or other means. Methods of producing appropriate vectors, transforming cells with those vectors, and identifying transformants are well known in the art and

25   are only briefly reviewed here (see, for example, Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

Prokaryotic cells useful for producing the transformed cells of the invention include members of the bacterial genera *Escherichia* (e.g., *E. coli*), *Pseudomonas* (e.g., *P. aeruginosa*),

30   and *Bacillus* (e.g., *B. subtillus*, *B. stearothermophilus*), as well as many others well known and frequently used in the art. Prokaryotic cells are particularly useful for the production of large quantities of the proteins or peptides of the invention (e.g., naturally occurring or synthetic 3-

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OSTs, fragments of the 3-OSTs, fusion proteins of the 3-OSTs). Bacterial cells (e.g., *E. coli*)
may be used with a variety of expression vector systems including, for example, plasmids with the
T7 RNA polymerase/promoter system, bacteriophage λ regulatory sequences, or M13 Phage
regulatory elements. Bacterial hosts may also be transformed with fusion protein vectors which
5   create, for example, Protein A, lacZ, trpE, maltose-binding protein, poly-His tag, or glutathione-
S-transferase fusion proteins. All of these, as well as many other prokaryotic expression systems,
are well known in the art and widely available commercially (e.g., pGEX-27 (Amrad, USA) for
GST fusions).

   Eukaryotic cells and cell lines useful for producing the transformed cells of the invention
10 include mammalian cells (e.g., endothelial cells, mast cells, COS cells, CHO cells, fibroblasts,
hybridomas, oocytes, embryonic stem cells), insect cells lines (e.g., *Drosophila* Schneider cells),
yeast, and fungi. Eukaryotic cells are particularly useful for embodiments in which it is necessary
that the 3-OST proteins, or functional fragments thereof, be properly post-translationally modified
(e.g., *N*-glycosylated) because *N*-glycosylation of these proteins appears to be important to their
15 stability and/or activity. Currently preferred cells are mammalian cells and, in particular, COS-7
cells, CHO, cells, murine primary cardiac microvascular endothelial cells (CMiE), murine mast cell
line C57.1, human primary endothelial cells of umbilical vein (HUVEC), F9 embryonal carcinoma
cells, rat fat pad endothelial cells (RFPEC), L cells (e.g., murine LTA tk^- cells), and cells derived
from the transgenic animals of the invention.

20   To accomplish expression in eukaryotic cells, a wide variety of vectors have been
developed and are commercially available which allow inducible (e.g., LacSwitch expression
vectors, Stratagene, La Jolla, CA) or constitutive (e.g., pcDNA3 vectors, Invitrogen, Chatsworth,
CA) expression of 3-OST nucleotide sequences under the regulation of an artificial promoter
element. Such promoter elements are often derived from CMV or SV40 viral genes, although
25 other strong promoter elements which are active in eukaryotic cells can also be employed to
induce transcription of 3-OST nucleotide sequences. Typically, these vectors also contain an
artificial polyadenylation sequence and 3' UTR which can also be derived from exogenous viral
gene sequences or from other eukaryotic genes. These expression systems are commonly
available from commercial sources and are typified by vectors such as pcDNA3 and pZeoSV
30 (Invitrogen, San Diego, CA). As described below, the vector pcDNA3 has been successfully used
to cause expression of 3-OST-1 proteins in transfected COS-7 cells. Numerous expression
vectors are available from commercial sources to allow expression of any desired 3-OST

transcript in more or less any desired cell type, either constitutively or after exposure to a certain exogenous stimulus (e.g., withdrawal of tetracycline or exposure to IPTG).

Vectors may be introduced into the recipient or "host" cells by various methods well known in the art including, but not limited to, calcium phosphate transfection, strontium

5   phosphate transfection, DEAE dextran transfection, electroporation, lipofection, microinjection, ballistic insertion on micro-beads, protoplast fusion or, for viral or phage vectors, by infection with the recombinant virus or phage.

<u>Transgenic Animal Models</u>

The present invention also provides for the production of transgenic non-human

10   animal models in which wild type, allelic variant, chimeric, or antisense 3-OST sequences are expressed, or in which 3-OST sequences have been inactivated or deleted (e.g., "knock-out" constructs) or replaced with reporter or marker genes (e.g., "knock-in reporter construct"). The 3-OST sequences may be conspecific to the transgenic animal (e.g., murine sequences in a transgenic mouse) or transpecific to the transgenic animal (e.g. human sequence in a transgenic

15   mouse). In such a transgenic animal, the transgenic sequences may be expressed inducibly, constitutively or ectopically. Expression may be tissue-specific or organism-wide. Engineered expression of 3-OST sequences in tissues and cells not normally containing 3-OST gene products may cause novel alterations of heparan polysaccharide structure and lead to novel cell or tissue phenotypes. Ectopic or altered levels of expression of 3-OST sequences may alter cell, tissue

20   and/or developmental phenotypes. Transgenic animals are useful as models of thromboembolic and other disorders arising from defects in heparan sulfate biosynthesis or metabolism. Transgenic animals are also useful for screening compounds for their effects on HS biosynthesis mediated by 3-OSTs. Transgenic animals transformed with reporter constructs may be used to measure the transcriptional effects of small molecules, drugs, protein physiological mediators,

25   carbohydrate effectors, mimetic compounds or physical perturbations on the expression of 3-OST loci *in vivo*. The transgenic animals of the invention, may be used to screen such compounds for therapeutic utility.

Animal species suitable for use in the animal models of the present invention include, but are not limited to, rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and

30   non-human primates (e.g., Rhesus monkeys, chimpanzees). For initial studies, transgenic rodents (e.g., mice) are preferred due to their relative ease of maintenance and shorter life spans.

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Transgenic non-human primates may be preferred for longer term studies due to their greater similarity to humans and their higher cognitive abilities.

Using the a nucleic acid disclosed and otherwise enabled herein, there are now several available approaches for the creation of a transgenic animal. Thus, the enabled animal models include: (1) animals in which sequences encoding at least a functional fragment of a wild type 3-OST gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a minigene (i.e., a genetic construct of the 3-OST with the introns, if any, removed) or a large genomic fragment; (2) animals in which sequences encoding at least a functional fragment of a normal 3-OST gene have been recombinantly substituted for one or both copies of the animal's homologous 3-OST gene by homologous recombination or gene targeting; (3) animals in which one or both copies of one of the animal's homologous 3-OST genes have been recombinantly "humanized" by the partial substitution of sequences encoding the human homologue by homologous recombination or gene targeting; (4) animals in which sequences encoding 3-OST transcriptional elements linked to a reporter gene have replaced the endogenous 3-OST gene and transcriptional elements; (5) "knock-out" animals in which one or both copies of the animal's 3-OST sequences have been partially or completely deleted or have been inactivated by the insertion or substitution by homologous recombination or gene targeting of exogenous sequences (e.g., stop codons,); (6) animals in which additional genes related to the biosynthesis or metabolism of heparan sulfates have been altered (e.g., a murine transgenic in which all of the genes in the HS pathway have been humanized). These and other transgenic animals of the invention are useful as models of thromboembolic and other disorders arising from defects in heparan sulfate biosynthesis or metabolism. These animals are also useful for screening compounds for their effects on HS biosynthesis mediated by 3-OSTs.

To produce an animal model (e.g., a transgenic mouse), a wild type or allelic variant 3-OST sequence or a wild type or allelic variant of a recombinant nucleic acid encoding at least a functional fragment of a 3-OST is preferably inserted into a germ line or stem cell using standard techniques of oocyte or embryonic stem cell microinjection, or other form of transformation of such cells. Alternatively, other cells from adult organism may be employed. Animals produced by these or similar processes are referred to as transgenic. Similarly, if it is desired to inactivate or replace an endogenous 3-OST sequence, homologous recombination using oocytes, embryonic

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stem or other cells may be employed. Animals produced by these or similar processes are referred to as "knock-out" (inactivation) or "knock-in" (replacement) models.

For oocyte injection, one or more copies of the recombinant DNA constructs of the present invention may be inserted into the pronucleus of a just-fertilized oocyte. This oocyte is

5   then reimplanted into a pseudo-pregnant foster mother. The liveborn animals are screened for integrants using analysis of DNA (e.g., from the tail veins of offspring mice) for the presence of the inserted recombinant transgene sequences. The transgene may be either a complete genomic sequence introduced into a host as a YAC, BAC or other chromosome DNA fragment, a cDNA with either the natural promoter or a heterologous promoter, or a minigene containing all of the

10   coding region and other elements found to be necessary for optimum expression.

To create a transgene, the target sequence of interest (e.g., wild type or allelic variant 3-OST sequences) are typically ligated into a cloning site located downstream of some promoter element which will regulate the expression of RNA from the sequence. Downstream of the coding sequence, there is typically an artificial polyadenylation sequence. An alternative approach

15   to creating a transgene is to use an exogenous promoter and regulatory sequences to drive expression of the transgene. Finally, it is possible to create transgenes using large genomic DNA fragments such as YACs which contain the entire desired gene as well as its appropriate regulatory sequences.

Animal models may be created by targeting endogenous 3-OST sequence in order to

20   alter the endogenous sequence by homologous recombination. These targeting events can have the effect of removing endogenous sequence (knock-out) or altering the endogenous sequence to create an amino acid change associated with human disease or an otherwise abnormal sequence (e.g., a sequence which is more like the human sequence than the original animal sequence) (knock-in animal models). A large number of vectors are available to accomplish this and

25   appropriate sources of genomic DNA for mouse and other animal genomes to be targeted are commercially available from companies such as GenomeSystems Inc. (St. Louis, Missouri, USA). The typical feature of these targeting vector constructs is that 2 to 4 kb of genomic DNA is ligated 5' to a selectable marker (e.g., a bacterial neomycin resistance gene under its own promoter element termed a "neomycin cassette"). A second DNA fragment from the gene of

30   interest is then ligated downstream of the neomycin cassette but upstream of a second selectable marker (e.g., thymidine kinase). The DNA fragments are chosen such that mutant sequences can be introduced into the germ line of the targeted animal by homologous replacement of the

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endogenous sequences by either one of the sequences included in the vector. Alternatively, the sequences can be chosen to cause deletion of sequences that would normally reside between the left and right arms of the vector surrounding the neomycin cassette. The former is known as a knock-in, the latter is known as a knock-out.

5   Retroviral infection of early embryos can also be done to insert the recombinant DNA constructs of the invention. In this method, the transgene (e.g., a wild type or allelic variant 3-OST sequence) is inserted into a retroviral vector which is used to directly infect embryos (e.g., mouse or non-human primate embryos) during the early stages of development to generate partially transgenic animals, some of which bear the transgenes in germline cells.

10   Alternatively, homologous recombination using a population of stem cells allows for the screening of the population for successful transformants. Once identified, these can be injected into blastocysts, and a proportion of the resulting animals will show germline transmission of the transgene.

           Techniques of generating transgenic animals, as well as techniques for homologous

15   recombination or gene targeting, are now widely accepted and practiced. A laboratory manual on the manipulation of the mouse embryo, for example, is available detailing standard laboratory techniques for the production of transgenic mice (69).

           Finally, equivalents of transgenic animals, including animals with mutated or inactivated 3-OST sequences may be produced using chemical or x-ray mutagenesis of gametes, followed by

20   fertilization. Using the isolated a nucleic acid disclosed or otherwise enabled herein, one of ordinary skill may more rapidly screen the resulting offspring by, for example, direct sequencing, SSCP, RFLP, PCR, or hybridization analysis to detect mutants, or Southern blotting to demonstrate loss of one allele by dosage.

Identifying Modulators of 3-OST Expression

25   In another set of embodiments, the present invention provides isolated nucleic acids comprising a genetic regulatory sequences of a 3-OST gene operably joined to a marker gene. Such regulatory sequences include 5' untranslated regions such as promoter and operator sequences. The 5' regulatory sequences of the human 3-OST-4 gene (as well as coding regions) are disclosed herein as SEQ ID NO: 16. Such regulatory regions may be used to transform host

30   cells, which are useful in methods of identifying compounds capable of modulating the expression of the 3-OST gene. Thus, in such methods, a candidate compound is contacted with a host cell transformed with a marker gene operably joined to the 3-OST regulatory regions, and changes in

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expression of the marker gene are indicative of the ability of the candidate compound to modulate 3-OST expression. Such methods may also be performed using the transgenic animals of the invention.

Substantially Pure Proteins

5   In one aspect, the present invention provides substantially pure preparations of 3-OST proteins. In preferred embodiments, the 3-OST proteins are 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4 or ce3-OST proteins. In particularly preferred embodiments, the 3-OST proteins are those disclosed as SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, or SEQ ID NO 15. As shown in the examples below, nucleic

10   acids encoding all or a portion of one mammalian 3-OST protein may be used to isolate homologues in other species by standard techniques known to those of ordinary skill in the art. Thus, the present invention also enables substantially pure protein preparations of 3-OST proteins of other mammalian species including, for example, rats, goats, sheep, cows, pigs, and non-human primates. Similarly, the isolated nucleic acids disclosed herein may be used to screen additional

15   human or other mammalian genetic libraries (e.g., genomic or cDNA libraries) to identify allelic variants of the particularly disclosed sequences. Thus, the present invention also enables substantially pure protein preparations of human and other mammalian 3-OST allelic variants.

         In another aspect, the present invention provides 3-OST protein variants in which conservative substitutions have been made for certain residues, or chimeric 3-OST proteins in

20   which the sequences of various 3-OST proteins have been mixed, to produce non-naturally occurring variants which retain 3-O-sulfotransferase activity. Conservative substitutions are preferably made in those regions of the proteins which are already known to vary amongst the human and murine sequences (see Figure 1) or between the 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B 3-OST-4, and ce3-OST proteins (see, e.g., Figure 2). Substitutions are to be avoided in

25   those areas which have been implicated in catalysis (see above). Chimeric 3-OST proteins may be made using the disclosed sequences as reference sequences, and these chimeras may also be subjected to conservative substitutions as described above. In addition, based upon the homologies of the 3-OST proteins to other glucosaminyl sulfotransferases (e.g., 2-OST, NST-1, NST-2), one of ordinary skill in the art may produce chimeric 3-OSTs using those proteins as

30   reference sequences (see, e.g., Figure 2).

         In preferred embodiments, the 3-OST proteins have at least 60%, , preferably at least 70%, and more preferably at least 80% amino acid sequence similarity to the mammalian 3-OST

sequences particularly disclosed herein, and retain 3-O-sulfotransferase activity. Most preferably, the sequences have at least 90% or 95% amino acid sequence similarity to the disclosed reference sequences. Such sequences may be routinely produced by those of ordinary skill in the art, and 3-O-sulfotransferase activity may be tested by routine methods such as those disclosed herein.

5   The substantially pure proteins of the present invention may be joined to other polypeptide sequences for use in various applications. Thus, for example, the proteins of the invention may be joined to one or more additional polypeptides so as to form a fusion protein, as is commonly known in the art and as described in the examples below. The additional polypeptides may be joined to the N-terminus, C-terminus or both termini of the 3-OST protein. Such fusion proteins

10   may be particularly useful if the additional polypeptide sequences are easily identified (e.g., by providing an antigenic determinant) or easily purified (e.g., by providing a ligand for affinity purification).

      In another aspect, the substantially pure 3-OST proteins of the present invention may comprise only a portion or fragment of the amino acid sequence of a complete mammalian 3-OST

15   protein. For example, as described above, the 3-OST-1 proteins comprise a twenty amino acid signal sequence which is removed post-translationally to yield the mature proteins. In some instances (e.g., when employing 3-OST-1 proteins *in vitro*), it may be preferable to employ only the mature protein or a minimal fragment retaining 3-O-sulfotransferase activity. In addition, the four C-terminal residues of 3-OST-1 may be involved in localization of the protein within the

20   Golgi apparatus. In some instances (e.g., when employing 3-OST-1 proteins *in vitro*), it may be preferable to employ a 3-OST-1 protein which does not include these residues, as they will be unnecessary for *in vitro* function. As described above, an approximately 260 amino acid portion of the 3-OST proteins includes the catalytically active region and, therefore, it may be preferable to employ a 3-OST protein which includes only this functional fragment which retains 3-O-

25   sulfotransferase activity. Thus, in certain preferred embodiments, the present invention provides substantially pure 3-OST proteins including mature forms of a mammalian 3-OST-1 protein, C-terminally truncated forms, or minimal functional fragments thereof. In addition, as described above, these proteins may also comprise conservative substitution variants or chimeras of 3-OST proteins.

30   In another aspect, the present invention provides for substantially pure protein preparations which comprise a sequence of at least 6-12, preferably 10-16, more preferably 16-22 consecutive amino acid residues from any one of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6,

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SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 15. Such polypeptides have utility to raise antibodies to corresponding regions of the 3-OST proteins. In particular, an analysis of the amino acid sequences of the 3-OST proteins suggests that there are regions which will have particular utility in generating antibodies. Thus, in preferred embodiments, the

5   inventions provides antigenic 3-OST polypeptides selected from the group consisting of (a) residues 4-29, 144-152, 208-222, 31-42, 155-181, 72-94, 195-205, 278-293, 113-136, 56-66, 230-245, 257-263, 301-306, 267-272 and 101-107 of SEQ ID NO: 2; (b) residues 4-22, 140-148, 205-218, 68-90, 191-201, 274-289, 110-133, 51-62, 226-241, 253-259, 151-163, 168-181, 297-302, 27-34, 97-107 and 263-268 of SEQ ID NO: 4; (c) residues 18-44, 199-207, 114-123,

10   319-328, 250-275, 238-246, 128-143, 47-59, 83-98, 332-349, 178-186, 289-295, 310-316, 63-76, 4-9, 209-218, 170-176 and 300-305 of SEQ ID NO: 6; (d) residues 22-57, 236-256, 166-186, 151-161, 138-147, 77-85, 348-354, 87-94, 323-335, 360-366, 284-314, 217-224, 376-383, 4-20, 130-136, 67-73, 389-395 and 338-343 of SEQ ID NO: 8; (e) residues 221-241, 8-66, 151-171, 135-146, 333-339, 308-320, 345-351, 269-299, 202-209, 361-368, 86-100, 71-80, 115-129,

15   374-380 and 323-328 of SEQ ID NO: 10; and (f) residues 280-290, 321-364, 371-388, 211-231, 393-399, 310-316, 421-438, 405-411, 262-268 and 292-301 of SEQ ID NO: 12. Note that these polypeptides are listed in decreasing order of preference within in group (a) to (f). Preferred antigenic peptide sequences also include residues 218-231, 87-100, 167-180 and 275-288 of SEQ ID NO: 2, which have been successfully used to generate antibodies to m3-OST-1.

20   Thus, in another aspect, the present invention provides for antibodies and methods for making antibodies which selectively bind with the 3-OST proteins. These antibodies include monoclonal and polyclonal antibodies, as well as functional antibody fragments such as F(ab) and Fc.

            The proteins or peptides of the invention may be substantially purified by any of a variety
25   of methods selected on the basis of the properties revealed by their protein sequences. As shown in the examples below, and previously described (26), cells naturally expressing 3-OST-1 proteins secrete the protein when grown in culture, and the proteins may be isolated from the cell culture medium. The 3-OST-2, 3-OST-3A, 3-OST-3B and 3-OST-4 proteins, however, appear to include transmembrane domains. Thus, these proteins are not expected to be secreted at high

30   levels. Because the 3-OSTs are found in the Golgi apparatus and microsomal bodies of cells which naturally express them, a fraction of cells including these organelles may be isolated and the proteins may be extracted from this fraction by, for example, detergent solubilization.

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Alternatively the 3-OST proteins, fusion proteins, or fragments thereof, may be purified from cells transformed or transfected with expression vectors. For example, insect cells such as *Drosophila* Schneider cells and baculovirus expression systems may be employed with vectors such as pPBLUEBAC and pMELBAC (Stratagene, La Jolla, CA); yeast expression systems with vectors

5 such as pYESHIS Xpress vectors (Invitrogen, San Diego, CA); eukaryotic expression systems with vectors such as pcDNA3 (Invitrogen, San Diego, CA), which causes constitutive expression, or LacSwitch (Stratagene, La Jolla, CA) which is inducible; or prokaryotic expression systems with vectors such as pKK233-3 (Clontech, Palo Alto, CA). In the event that the protein or fragment localizes within microsomes derived from the Golgi apparatus, endoplasmic reticulum,

10 or other membrane containing structures of such cells, the protein may be purified from the appropriate cell fraction. Alternatively, if the protein does not localize within these structures, or aggregates in inclusion bodies within the recombinant cells (e.g., prokaryotic cells), the protein may be purified from whole lysed cells or from solubilized inclusion bodies by standard means.

Purification can be achieved using standard protein purification procedures including, but

15 not limited to, affinity chromatography, gel-filtration chromatography, ion-exchange chromatography, high-performance liquid chromatography (RP-HPLC, ion-exchange HPLC, size-exclusion HPLC), high-performance chromatofocusing chromatography, hydrophobic interaction chromatography, immunoprecipitation, or immunoaffinity purification. Gel electrophoresis (e.g., PAGE, SDS-PAGE) can also be used to isolate a protein or peptide based on its molecular

20 weight, charge properties and hydrophobicity.

A 3-OST protein, or a fragment thereof, may also be conveniently purified by creating a fusion protein including the desired 3-OST sequence fused to another peptide such as an antigenic determinant (e.g., from Protein A, see below) or poly-His tag (e.g., QIAexpress vectors, QIAGEN Corp., Chatsworth, CA), or a larger protein (e.g., GST using the pGEX-27 vector

25 (Amrad, USA) or green fluorescent protein using the Green Lantern vector (GIBCO/BRL. Gaithersburg, MD). The fusion protein may be expressed and recovered from prokaryotic or eukaryotic cells and purified by any standard method based upon the fusion vector sequence. For example, the fusion protein may be purified by immunoaffinity or immunoprecipitation with an antibody to the non-3-OST portion of the fusion or, in the case of a poly-His tag, by affinity

30 binding to a nickel column. The desired 3-OST protein or fragment can then be further purified from the fusion protein by enzymatic cleavage of the fusion protein. Methods for preparing and

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using such fusion constructs for the purification of proteins are well known in the art and numerous kits are now commercially available for this purpose.

Currently preferred methods for small scale purification of 3-OST-1 proteins from the media of LTA cells grown in culture may be found in Liu et al. (26), and methods for purification

5   of 3-OSTs produced recombinantly in COS-7 cells, CHO cells, murine primary cardiac microvascular endothelial cells (CME), murine mast cell line C57.1, and human primary endothelial cells of umbilical vein (HUVEC) may be found in the examples below. These methods may also be adapted for use with other cell and expression systems to obtain substantially pure 3-OST proteins.

10   In another aspect, the present invention provides for methods for producing the above-described proteins. Thus, in one set of embodiments, the isolated nucleic acids of the invention may be used to transform host cells or create transgenic animals. The proteins of the invention may then be substantially purified by well known methods including, but not limited to, those described in the examples below. Alternatively, the isolated nucleic acids of the invention may be

15   used in cell-free *in vitro* translation systems. Such systems are also well known in the art and include, but are not limited to, that described in the examples below.

Antibodies

The present invention also provides antibodies and methods of making antibodies, which will selectively bind to and, thereby, isolate or identify wild type and/or variant forms of the

20   3-OST proteins. The antibodies of the invention have utility as laboratory reagents for, inter alia, immunoaffinity purification of the 3-OSTs, immunoaffinity purification of 3-OST conjugates or complexes (e.g., 3-OST-AT, 3-OST-HS), Western blotting to identify cells or tissues expressing the 3-OSTs, and immunocytochemistry or immunofluorescence techniques to establish the cellular or extracellular location of the protein.

25   The antibodies of the invention may be generated using the entire 3-OST proteins of the invention or using any 3-OST epitope which is characteristic of that protein and which substantially distinguishes it from other host proteins. Such epitopes may be identified by comparing sequences of amino acid residues from a 3-OST sequence to computer databases of protein sequences from the relevant host. Preferably, the epitopes are chosen so as to be highly

30   immunogenic and specific.

In a preferred embodiment, the immunogen/epitope is a protein sequence of at least 6-12, preferably 10-16, more preferably 16-22 consecutive amino acid residues of the disclosed

OST genes. In particular, an analysis of the amino acid sequences of the 3-OST proteins suggests that there are regions which will have particular utility in generating antibodies. Thus, in preferred embodiments, the inventions provides antigenic 3-OST polypeptides.

3-OST immunogen preparations may be produced from crude extracts (e.g.,

5   microsomal fractions of cells expressing the proteins), from proteins or peptides substantially purified from cells which naturally or recombinantly express them or, for small immunogens, by chemical peptide synthesis. The 3-OST immunogens may also be in the form of a fusion protein in which the non-3-OST region is chosen for its adjuvant properties and/or the ability to either and/or facilitate purification. As used herein, a 3-OST immunogen shall be defined as a

10   preparation including a peptide comprising at least 4-8, and preferably at least 9-15 consecutive amino acid residues of the 3-OST proteins or nucleic acids encoding such a peptide coupled with transcriptional elements, as disclosed or otherwise enabled herein. Therefore, any 3-OST derived polypeptide or protein sequences which are employed to generate antibodies to the 3-OSTs should be regarded as 3-OST immunogens.

15   The antibodies of the invention may be polyclonal or monoclonal, or may be antibody fragments, including Fab fragments, $F(ab')_2$, and single chain antibody fragments. In addition, after identifying useful antibodies by the method of the invention, recombinant antibodies may be generated, including any of the antibody fragments listed above, as well as humanized antibodies based upon non-human antibodies to the 3-OST proteins. In light of the present disclosures of 3-

20   OST proteins, as well as the characterization of other 3-OSTs enabled herein, one of ordinary skill in the art may produce the above-described antibodies by any of a variety of standard means well known in the art. For an overview of antibody techniques, see Antibody Engineering, 2nd Ed., Borrebaek, ed., Oxford University Press, Oxford (1995).

As a general matter, monoclonal anti-3-OST antibodies may be produced by first

25   injecting a mouse, rabbit, goat or other suitable animal with a 3-OST immunogen in a suitable carrier or diluent. As above, carrier proteins or adjuvants may be utilized and booster injections (e.g., bi- or tri-weekly over 8-10 weeks) are recommended. After allowing for development of a humoral response, the animals are sacrificed and their spleens are removed and resuspended in, for example, phosphate buffered saline (PBS). The spleen cells serve as a source of lymphocytes,

30   some of which are producing antibody of the appropriate specificity. These cells are then fused with an immortalized cell line (e.g., myeloma), and the products of the fusion are plated into a number of tissue culture wells in the presence of a selective agent such as HAT. The wells are

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serially screened and replated, each time selecting cells making useful antibody. Typically, several
screening and replating procedures are carried out until over 90% of the wells contain single
clones which are positive for antibody production. Monoclonal antibodies produced by such
clones may be purified by standard methods such as affinity chromatography using Protein A
5   Sepharose, by ion-exchange chromatography, or by variations and combinations of these
techniques.

The antibodies of the invention may be labeled or conjugated with other compounds or
materials for diagnostic and/or therapeutic uses. For example, they may be coupled to
radionuclides, fluorescent compounds, or enzymes for imaging or therapy, or to liposomes for the
10   targeting of compounds contained in the liposomes to a specific tissue location.

Assays for Drugs Which Affect 3-OST Expression

In another series of embodiments, the present invention provides assays for identifying
small molecules or other compounds which are capable of inducing or inhibiting the expression of
the 3-OST genes and proteins. The assays may be performed *in vitro* using non-transformed
15   cells, established cell lines, or the transformed cells of the invention, or *in vivo* using normal non-
human animals or the transgenic animal models of the invention.

In particular, the assays may detect the presence of increased or decreased expression
of nucleic acids under the transcriptional control of 3-OST promoter and regulatory sequences on
the basis of increased or decreased mRNA expression (using, e.g., the nucleic acid probes
20   disclosed and enabled herein), increased or decreased levels of protein products encoded for such
nucleic acids (using, e.g., the anti-3-OST antibodies disclosed and enabled herein), or increased or
decreased levels of activity of such a protein (e.g., β-galactosidase or luciferase).

Thus, for example, one may culture cells known to express a particular 3-OST, or
recombination modified to express at least a functional fragment or epitope of 3-OST protein
25   under the transcriptional control of 3-OST promoter and add to the culture medium one or more
test compounds. After allowing a sufficient period of time (e.g., 0-72 hours) for the compound to
induce or inhibit the expression of the 3-OST, any change in levels of expression from an
established baseline may be detected using any of the techniques well known in the art. Using the
nucleic acid probes and /or antibodies disclosed and enabled herein, detection of changes in the
30   expression of a 3-OST, and thus identification of the compound as an inducer or inhibitor of 3-
OST expression, requires only routine experimentation. For example, one may assay for 3-OST
activity by measuring the conversion of HS^{Inact} into HS^{Act} by methods known in the art (70).

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In other embodiments, a recombinant assay is employed in which a reporter gene is operably joined to 3-OST promoter and regulatory sequences so as to be under the transcriptional control of these sequences. The reporter gene may be any gene which encodes a transcriptional or transitional product which is readily assayed or which has a readily determinable affect or

5   phenotype. Preferred reporter genes are those encoding enzymes with readily detectable activity, including without limitation β-galactosidase, green fluorescent protein , alkaline phosphatase, or luciferase is operably joined to the 5' regulatory regions of a 3-OST gene. The 3-OST regulatory regions, may be readily isolated and cloned by one of ordinary skill in the art in light of the present disclosure of the coding regions of these genes. The reporter gene and regulatory regions are

10 joined in-frame (or in each of the three possible reading frames) so that transcription and translation of the reporter gene may proceed under the control of the 3-OST regulatory elements. The recombinant construct may then be introduced into any appropriate host cell as described herein. The transformed cells may be grown in culture and, after establishing the baseline level of expression of the reporter gene, test compounds may be added to the medium. The ease of

15 detection of the expression of the reporter gene provides for a rapid, high through-put assay for the identification of inducers and inhibitors of the 3-OST gene.

Compounds identified by this method will have potential utility in modifying the expression of the 3-OST genes *in vivo*. These compounds may be further tested in the animal models disclosed and enabled herein to identify those compounds having the most potent *in vivo*

20 effects.

Methods for Heparan Modification

In another aspect, the present invention provides methods for 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan or proteoglycan polysaccharides in which the polysaccharides include a polysaccharide sequence of GlcA→GlcNS ±6S. These methods

25 comprise contacting the GlcA→GlcNS ±6S-containing polysaccharide preparation with 3-OST protein in the presence of a sulfate donor under conditions which permit the 3-OST to convert the GlcA→GlcNS ±6S sequence to GlcA→GlcNS 3S ±6S. In particular embodiments, the GlcA→GlcNS ±6S sequence comprises a part of an HS^{act} precursor sequence (i.e., GlcA→GlcNS ±6S→IdoA 2S→ GlcNS ±6S or IdoA→GlcNAc 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS

30 6S) or a part of an HS^{inact} precursor sequence (i.e., IdoA→GlcNS 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S; IdoA→GlcNAc→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;

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IdoA→GlcNS→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S; IdoA→GlcNAc

6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS or IdoA→GlcNS 6S→GlcA→GlcNS ±6S→IdoA

2S→ GlcNS). Conversion of the HS^{act} precursor pool to HS^{act} increases the fraction with AT-

binding activity and is particularly useful in the production of anticoagulant heparan sulfate

products. Thus, in another embodiment, the present invention provides for means of enriching the

AT-binding fraction of a heparan sulfate pool by contacting the polysaccharide preparation with

3-OST protein in the presence of a sulfate donor under conditions which permit the 3-OST HS^{act}

conversion activity. In preferred embodiments, the sulfate donor is 3'-phospho-adenosine 5'-

phosphosulfate (PAPS).

Methods of Partially Sequencing Complex Polysaccharides

In another aspect, the present invention provides methods for partially sequencing

complex polysaccharides such as heparan sulfates (HS) or other glycosaminoglycans (GAGs). In

these methods, a pool of polysaccharides which includes sequences which may be 3-O-sulfated is

contacted with a 3-OST protein in the presence of a sulfate donor (e.g., PAPS) under conditions

which permit sulfation by 3-OST. The treated polysaccharides are then subjected to degradation

by enzymes which degrade polysaccharides in a sequence-specific manner (e.g., polysaccharide

lyases; heparinase I, II or III) and the size profile of the resulting fragments is determined. An

identical pool which has not been treated with 3-OST is similarly cleaved by the same enzymes

and a size profile determined. Changes in the size profiles indicate that 3-OST activity has

modified the saccharide units so as to prevent (or permit) cleavage at sites which previously were

(or were not) cleaved. Thus, comparison of the profiles will indicate positions at which the target

sequences for 3-OST activity are present and provide a partial polysaccharide sequence.

In another embodiment, the sequence of complex polysaccharides such as HS or GAG

may be partially determined using sequence specific polysaccharide affinity fractionation. To this

end, 3-OST proteins which lack enzymatic function can be identified or produced (e.g., altering or

deleting a portion of the catalytic ST domain by site-directed or deletion mutagenesis). These

inactive forms will bind GAGs in a sequence dependent manner. For example, the 3-OST-1

protein normally, minimally, binds a GAG sequence containing GlcA-GlcNS ±6S. When the

active site of this protein is neutralized, the k_d of the protein for these sequences will be relatively

unaffected. This reagent will allow sequence-specific saccharide affinity fractionation from

complex mixtures of GAGs. The purified structures can be degraded in a step-wise fashion with

exolytic, endolytic enzymes and/or nitrous acid, and the resulting degradation products can be

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compared to standard compounds of known structure. This method will allow the quantitation and characterization of known structures contained within unknown complex polysaccharide samples.

In another embodiment, partial sequence can be obtained using the 3-OSTs of the invention or other heparan sulfate sequence specific binding ligands as protective groups prior to treating the HS or GAG with modifying agents that detectably alter the HS or GAG. Useful protective groups include catalytically inactive enzymes, chimeric enzymes and small molecule ligands with identified sequence binding specificities. The protecting group is contacted with the heparan or other glycosaminoglycans (GAGs), and the resultant complex is treated with one or more modifying agents. Useful modifying agents include catalytically active heparan lyases, sulfotransferases, N-deacetylases, epimerases, or chimeric proteins of the invention. In embodiments where multiple protecting groups and/or modifying reagents in are used in combination, the sample is first contacted with the protective group, then each modifying reagent may be with contacted with the protected polysaccharide, either simultaneously or in turn. The protective group will interfere with the ability of a chemically modifying agent to interact with, attach to and/or cleave specific GAG sequence motifs. The sample can then be analyzed for ligand-specific protection and/or cleavage to elucidate the sequence of the original GAG using separation and/or quantitation using methods known in the art.

In some embodiments, as a preliminary step, full length heparans and GAG oligomers can be fractionated over an immobilized affinity ligand immobilized at their reducing ends *via* hydrazide chemistry. The fraction of GAG captured by the immobile phase permits a quantitation of the mass or total percent of the target sequence (out of total GAG.) Thus, unique heparan or other GAG structures may be concentrated and/or specifically eluted for further analysis.

One useful method for the detection binding is the Biomolecular Interaction Assay or "BIAcore" system developed by Pharmacia Biosensor and described in the manufacturer's protocol (LKB Pharmacia, Sweden). In light of the present disclosure, one of ordinary skill in the art is now enabled to employ this system, or a substantial equivalent, to identify proteins or other compounds having sequence-specific HS or GAG binding capacity, or HS or GAGs sequences having 3-OST binding capacity. Such systems utilize surface plasmon resonance, an optical phenomenon that detects changes in refractive indices. A sample of interest is passed over an immobilized ligand (e.g., a 3-OST fusion protein or specific GAG) and binding interactions are registered as changes in the refractive index.

Examples

Cell Lines and Cell Culture

The clonal L cell line LTA (35, 41), the generation of clone 33, an LTA transfectant that over-expresses the ryudocan$_{12CAS}$ cDNA (33), a rapidly growing revertant of clone 33, L-33$^+$ (26), and RFPEC, an immortalized line derived from rat fat-pad endothelial cells (8) have previously been described. Primary mouse neonatal endothelial cells from the cardiac microvasculature of day 3-5 neonates (CME cells) (from Dr. Jay Edelberg, MIT/Beth Israel Hospital) and COS-7 cells (ATCC) were employed. Primary human umbilical cells (HUVEC) were maintain according to the supplier's (Clonetics Inc.) protocol. Unless otherwise stated, all cell lines were maintained in logarithmic growth by subculturing biweekly in Dulbecco's modified Eagle medium (Life Technologies, Inc.) containing 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 units/ml penicillin at 37 °C under 5% CO_2 humidified atmosphere, as previously described (42). Exponentially growing cultures were generated by inoculating 54,000 cells/cm^2 and incubating for two days, whereas post-confluent cultures were produced by inoculating 250,000 cells/cm^2 and allowing growth for 10 days with medium exchanges on days 4, 7, 8, and 9.

Peptide Purification and Sequencing

The purification of mouse 3-OST-1 from L-33$^+$ has been previously described (26) and the final step 4 product was concentrated by reverse phase chromatography on a HP 1090 M system (Hewlett Packard) equipped with a C4 reverse phase HPLC column (250 x 2.1 mm, 300 Å pore size, 5 μm particle size) (Vydac, number 214TP52) equilibrated in 1.6% acetonitrile (v/v), 0.1% TFA (v/v). After application of sample, the reverse phase matrix was washed with 60% acetonitrile, 0.1% TFA, and bound species were eluted with 78.4% acetonitrile, 0.1% TFA. Samples of 1.5 or 3 μg, from two independent purifications, were digested with 0.15 or 0.3 μg, respectively, of endopeptidase Lys-C (Waco) in a reaction volume of 100 μl containing 1% RTX100 (Calbiochem), 10% acetonitrile and 100 mM Tris-HCl pH 8.0, at 37 °C for ~16 h (43). Digestion products were chromatographed on an HP 1090 M system (Hewlett Packard) equipped with the above described C4 reverse phase HPLC column equilibrated in 98% Buffer A (0.1% TFA (v/v))/2% Buffer B (80% acetonitrile (v/v)/0.85% TFA (v/v)). After application of digestion products, the reverse phase matrix was washed with 98% Buffer A/2% Buffer B, and bound species were eluted with linear gradients of Buffer B increasing to 37.5% over 60 min, to 75% over 30 min, and to 98% over 15 min (44). The eluate was monitored for absorbance at 210 and

280 nm, peptide peaks were individually collected and analyzed with a model 477A/120A Protein

Sequenator (Applied Biosystems). In addition, the NH$_2$-terminal sequence of 1 μg of

concentrated 3-OST-1 sample was directly determined.

Isolation of Mouse 3-OST-1 Clones

5   Isolation of Cytoplasmic and Poly(A)$^+$ RNA. Cytoplasmic RNA (17.5 mg) was isolated

from post-confluent cultures of LTA cells (12 flasks of 175 cm^2, ~1.6 x 10^9 cells) by a

modification of the procedure of Favaloro (45). Monolayers were twice washed with PBS, cells

were recovered by trypsinization and centrifugation (1000 x g for 2 min), and cell pellets were

washed by resuspension in PBS followed by centrifugation (1300 x g for 4 min). Cells were lysed

10   by vortexing for 30 sec in 12 ml of ice cold 50 mM Tris, pH 7.4, 140 mM NaCl, 5 mM EDTA,

1 % Triton X-100, 5 mM vanadium ribonucleoside complexes (Life Sciences Technologies),

samples were incubated on ice for 10 min and then vortexed for 1 min. Nuclei were pelleted by

centrifugation at 6000 x g for 10 min, the supernatant was mixed with an equal volume of 200

mM Tris, pH 7.4, 300 mM NaCl, 2% SDS, 25 mM EDTA, containing 200 μg/ml of proteinase K

15   (Boehringer Mannheim), and the mixture was incubated at 65 °C for 2 hr. Samples were

extracted twice against an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1), the

aqueous phase was combined with 0.7 volumes of isopropanol, cytoplasmic RNA was pelleted by

centrifugation at 3500 x g for 10 min, and was resuspended in 3.6 ml of 10 mM Tris, pH 7.4, 1

mM EDTA. Poly(A)$^+$ RNA (59 μg) was isolated from 16 mg of cytoplasmic RNA by two

20   sequential purifications against 100 mg of oligo(dT) cellulose (Life Sciences Technologies,

#15939-010) according to the manufacturer's specifications except that binding and wash buffers

contained 0.1 % SDS and LiCl was substituted for NaCl. The final eluate (1.5 ml) was extracted

against 1.5 ml of phenol/chloroform/isoamyl alcohol (25:24:1), the aqueous phase was then

adjusted to 100 mM LiCl and 260 mM NaCl, an equal volume of isopropanol was added, the

25   mixture was centrifuged at 15,000 x g for 30 min and the poly(A)$^+$ RNA pellet was recovered in

40 μl of diethyl pyrocarbonate treated water.

          PCR Cloning and Generation of a Mouse 3-OST-1 Probe. Degenerate PCR primers 1S,

2S, 2A, and 3A (described in Shworak et al. (1997) J. Biol. Chem. 272, in press) were obtained

from Bio Synthesis. First strand cDNA was generated in a 50 μl volume from 5 μg of LTA

30   poly(A)$^+$ RNA primed with oligo(dT) using an RT-PCR kit (Stratagene, La Jolla, CA) according

to the manufacturer's specifications. Touchdown PCR (46, 47) reactions (50 μl) contained 1 μl

of first strand cDNA, 25 pmol of each primer, 0.25 μl of AmpliTaq Gold (Perkin Elmer), 200 μM

of each dNTP and 1 x GeneAmp PCR buffer. Two distinct sets of touchdown PCR conditions were required to obtain optimal yields of product. For amplification with primers 1S and 2A, reactions were heated to 95 °C for 9 min, subjected to 20 cycles of 94 °C for 30 sec, and 68 °C for 1 min with a 0.5 °C reduction per cycle, followed by 20 cycles of 94 °C for 30 sec, 58 °C for

5   30 sec with a 0.5 °C reduction per cycle, and 75 °C for 30 sec, then 15 cycles of 94 °C for 30 sec, 55 °C for 10 sec, and ramping to 75 °C over 50 sec. Alternatively, for amplification with primers 1S and 3A or primers 2S and 3A, reactions were heated to 95 °C for 4 min, subjected to 47 cycles of 95 °C for 30 sec, and 69.5 °C for 2 min with 0.2 °C and 1 sec reductions per cycle, followed by 25 cycles of 95 °C for 30 sec, 60 °C for 15 sec, and ramping to 75 °C over 1 min.

10   Amplification products were purified as the retentate from centrifugal ultrafiltration against a 30,000 molecular weight cutoff membrane (Millipore, # SK1P343JO), then 200 ng of DNA was end polished with *Pfu* DNA polymerase and subcloned into pCR-Script Amp SK(+) (Stratagene, La Jolla, CA, #211188) according to the manufacturer's specifications. A resulting plasmid, pNWS182, contained the 1S/3A amplification product of 779 bp which was released by digestion

15   with *Eco*RI and *Sac*II, and isolated by low melting point agarose gel electrophoresis. A ^{32}P-labeled primer extension probe was then generated with a random primer labeling kit (Stratagene, La Jolla, CA, # 300385) by replacing the random primers with 5 μM of primer 3A.

Construction and Screening of an L Cell cDNA Library. Using the manufacturer's recommended conditions, an oligo(dT)-primed λ Zap Express cDNA library (Stratagene, La Jolla,

20   CA, # 200451) was generated from 5 μg of LTA poly(A)$^+$ RNA which had been pretreated with methylmercury hydroxide. About 1.5 x 10^6 primary recombinants were plaque amplified by infection into *E. coli* XL1-Blue MRF'. From the amplified library, 1.3 x 10^6 plaques were transferred to Colony/Plaque Screen (Du Pont-New England Nuclear) and screened with the above described ^{32}P-labeled probe specific for 3-OST-1. Hybridizations were performed at 42 °C

25   in 1.7 x SSC, 8.3% dextran sulfate, 42% formamide, 0.8% SDS and filters were washed twice with 2 x SSC, 1% SDS for 30 min at 65 °C. Positive clones were plaque purified and then *in vivo* excised into pBK-CMV based phagemids by infection with ExAssist helper phage followed by transduction of filamentous phage particles into *E. coli* XLOLR.

Isolation of Human 3-OST-1 cDNA Clones

30   The National Center for Biotechnology Information data bank of I.M.A.G.E. Consortium (LLNL) expressed sequence tag cDNA clones (48) was probed with the deduced mouse 3-OST-1

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amino acid sequence to reveal three partial length species. I.M.A.G.E. Consortium CloneID-220372 (accession numbers H86812 and H86876) was from the retinal library of Soares (N2b4HR), whereas clones 301725 (accession numbers N90867 and W16558) and 301726 (accession numbers N90856 and W16555) were from the fetal lung library of Soares (NbHL19W

5   ) and were obtained from the TIGR/ATCC Special Collection (ATCC). The *EcoRI/Not*I insert of clone 220372 was ^{32}P labeled by random priming and used to screen 5 x 10^5 plaques from a λ TriplEx Brain cDNA library (Clontech, Palo Alto, CA), as described above. Positive plaques were purified, TriplEx based plasmids were *in vivo* excised according to the manufacturer's protocol, and were sequenced as described below.

10   Characterization of Mouse and Human 3-OST-1 cDNA Clones

        The 5' and 3' regions of all partial and full length clones were enzymatically sequenced from flanking primer sites of the respective cloning vectors. For full length clones the remaining sequence of both strands was obtained with internally priming oligonucleotides. Automated fluorescence sequencing was performed with Perkin Elmer Applied Biosystems Models 373A and

15   477 DNA sequencers. Each reaction typically yielded 400 to 600 bases of high quality sequence. cDNA sequence files were aligned and compiled with the program Sequencher 3.0 (Gene Codes Corp.). All additional manipulations were performed with the University of Wisconsin Genetics Computer Group sequence analysis software package. Sequence comparison searches were performed on the databases of GenBank, EMBL, DDBJ, PDB, SwissProt, PIR, and dbEST.

20   Expression of 3-OST-1 cDNAs

        Construction of Expression Plasmids. The plasmid pCMV-3-OST contains the mouse 3-OST-1 cDNA, an *EcoRI/Xho*I fragment from pNWS228, inserted between the CMV promoter and the bovine growth hormone polyadenylation signal of *EcoRI/Xho*I digested and phosphatase treated pcDNA3 (Invitrogen). The plasmid pCMV-ProA3-OST is of similar structure, except the

25   first 26 amino acid of 3-OST-1 are replaced with 291 amino acids encoding a fusion protein of the transin leader sequence followed by Protein A and a factor Xa cleavage site. pCMV-ProA-3-OST was generated by ligating a *Bam*HI/*Sma*I fragment containing the Protein A region from pRK5F10PROTA (49), and an *Xma*I (end-filled with T$_4$ polymerase)/*Xho*I fragment containing most of the mouse 3-OST-1 cDNA from pNWS228, into *Bam*HI/*Xho*I digested and phosphatase

30   treated pcDNA3 (Invitrogen). The *in vitro* transcription plasmid, pNWS237, contains a T3 promoter site 5' of the human 3-OST-1 cDNA and was constructed by inserting complementary oligonucleotides (Bio Synthesis) into the *Eco*RI site of the TriplEx based plasmid, pJL30.

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Transient Expression of the Mouse 3-OST-1 cDNA in COS-7 Cells. For each expression
construct, three 175 cm^2 flasks were seeded with 3.6×10^6 COS-7 cells, 6 h later the medium was
exchanged with DMEM containing 10% Nu-Serum (Life Technologies, Inc.) with 100 µg/ml
streptomycin and 100 units/ml penicillin, and cells were grown for an additional day. Monolayers

5   were washed with PBS then incubated at 37 °C for 2.5 h with 10 ml/flask of freshly prepared
DMEM containing 235 µg/ml DEAE-dextran (M.W. 500,000, Pharmacia), 9.5 mM Tris-HCl, pH
7.4, 0.9 mM chloroquine-diphosphate (Sigma), and 3 µg/ml of the appropriate pcDNA3 based
expression plasmid. Monolayers were then exposed to freshly prepared 10% DMSO in PBS for
1.5 min, washed twice with nonsupplemented DMEM, fed 30 ml/flask of DMEM containing 10%

10 fetal bovine serum, 100 µg/ml streptomycin, and 100 units/ml penicillin, and cells were grown for
an additional day. Monolayers were washed with PBS, then cells were grown in 40 ml/flask
Serum-Free Medium (DMEM containing 25 mM HEPES, pH 8.0, 1% Nutridoma SP (Boehringer
Mannheim) (v/v), an additional 2 mM glutamine, 10 ng/ml biotin (Pierce), 100 µg/ml
streptomycin, 100 units/ml penicillin, and 1 x of a previously described Trace Metal Mix (26)) for

15 24 h. COS-cell conditioned Serum-Free Medium was harvested, debris was removed by
centrifugation at 1,000 x g for 10 min follrwed by filtration through a 0.45 µm membrane, then
samples were either immediately processed or were snap frozen with liquid nitrogen and stored at
-80 °C. Occasionally, conditioned medium from a second incubation of 8-24 h was also collected.

Purification of Wild-type and Protein A Tagged Mouse Recombinant 3-OST-1. Wild-type

20 mouse recombinantly expressed 3-OST-1 enzyme (r3-OST-1) was purified, at 4 °C, from 240 ml
of freshly generated Serum-Free Medium conditioned by COS-7 cells transfected with pCMV-3-
OST. The medium was adjusted to pH 8.0, mixed with an equal volume 2% glycerol, then loaded
(25 ml/h) onto a heparin-AF Toyopearl-650M column (0.8 x 5.7 cm) (TosoHaas,
Montgomeryville, PA) equilibrated in 50 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1% glycerol (v/v)

25 (Buffer C). The column was washed with 20 ml of Buffer C at a flow rate of 0.8 ml/min, then
with 20 ml of 150 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1% glycerol (v/v) at a flow rate of 0.5
ml/min, and protein was eluted at a flow rate of 0.25 ml/min with a 20 ml linear NaCl gradient
extending from 150 mM to 750 mM NaCl in Buffer C. The fractions exhibiting HS^{act} conversion
activity (approximately 4 ml) were pooled, brought to a final concentration of 0.6% CHAPS

30 (w/v) (Sigma) and dialyzed for 16 h against 4 l of 25 mM MOPS (3-[N-morpholino]
propanesulfonic acid) (Sigma), pH 7.0, 1% glycerol (v/v), 0.6% CHAPS (w/v) (MCG buffer)
containing 50 mM NaCl. The dialysate was applied to a 3',5'-ADP-agarose column (0.8 x 1.2 cm,

3.7 mmol of 3',5'-ADP/ml of gel) (Sigma) and eluted as previously described (26). The fractions containing HS^{act} conversion activity were pooled (approximately 4 ml), aliquoted, frozen in liquid nitrogen and stored at -80 °C.

5   Protein A tagged mouse r3-OST-1 was purified, at 4 °C, from 155 ml of previously frozen Serum-Free Medium conditioned by COS-7 cells transfected with pCMV-ProA3-OST. IgG agarose beads (310 µl of a 50/50 slurry; Sigma) were gently stirred with the conditioned medium for 3h, recovered by centrifugation at 2,000 x g for 10 min, and washed twice with 1 ml of MCG containing 250 mM NaCl to remove nonspecifically bound protein. Protein A fusion-protein was eluted from the beads with two sequential 30 min incubations in 100 µl of 50 mM sodium acetate,

10   pH 4.5, 150 mM NaCl, 0.6% CHAPS and 1% glycerol. The pooled eluates were combined with an equal volume of 500 mM MOPS, pH 7.0, 0.6% CHAPS, and 1% glycerol, then aliquoted, frozen in liquid nitrogen and stored at -80 °C.

Retroviral Transduction of CHO and MNE Cells with 3-OST-1

    Plasmid retrovirus vector construction. A retroviral transduction system was used to

15   transduce CHO cells and mouse neonatal endothelial (MNE) cells. This system may serve as a model for in vivo transduction for use in gene therapy.

    The retrovirus backbone plasmid pMSCV-PGK-EGFP is a derivative of pMSCVpac a (Dr. Robert Hawley University of Toronto.) The puromycin acetyl transferase gene cassette in pMSCVpac was removed and replaced with an Enhanced GFP (Dr. David Baltimore MIT). The

20   pMSCV-PGK-GFP vector was assembled by digestion of the plasmid with HindIII and ClaI, followed by treatment with Klenow fragment. The EGFP cistron 720 bp fragment was derived from the digestion of pMSCV-EGFPpac with EcoRI, and blunting with the Klenow fragment. The EGFP blunt-ended fragment was then ligated into the blunt-ended pMSCV vector. The resulting plasmids were tested for proper orientation by restriction analysis. The reporter virus,

25   pMSCVPLAP, is designed to express the wild type human placental alkaline phosphatase (PLAP) transcribed from the 5' LTR. pMSCV-SEAP-PGK-EGFP was made by cloning the secreted alkaline phosphatase (SEAP) BgIII and HpaI 1.723 kb fragment from pSEAP2-basic plasmid (Clontech, Palo Alto, CA) into the BgIII and HpaI cut pMSCV-PGKEGFP vector. pCMV3-OST was digested with BgIII and XhoI to release the wild type mouse 3-OST-1 cDNA. The 1.623 kb

30   3-OST-1 cDNA fragment was cloned into the BgIII and XhoI sites in pMSCV-PGK-EGFP. The occurrence of the insert of interest present in the correct orientation was ascertained by restriction

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analysis. All plasmid DNA prepared for transfection was made with the Invitrogen SNAP-MIDI kits according to the manufacturer's directions.

Cells and cell culture. Dulbecco's modified Eagle medium (DMEM), F-12 Ham's medium and penicillin/streptomycin, 0.25% trypsin, 10 mM EDTA, were obtained from Life Technologies,

5   Inc., GIBCO-BRL (Gaithersburg, MD). The PHOENIX ecotropic retroviral packaging cell line (ATCC #SD 3444) was grown in DMEM, 10% heat-treated fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS), 100 units/ml penicillin, 100 µg/ml. PHOENIX cells were subcultured three times weekly at a split ratio of approximately 1:8 in a 37 °C humidified, 5.0% CO_2 incubator. CHOK1 ATCC CCL 61 cells (CHO) were grown in F-12 medium supplemented with

10 10% fetal bovine serum, and 100 units/ml penicillin, 100 µg/ml in a 37 °C humidified, 5.0% CO_2 incubator. CHO cells were subcultured three times weekly at a split ratio of approximately 1:4 in a 37 °C humidified, 5.0% CO_2 incubator. 1×10^6 CHO cells were transfected with 10 µg of pcB7-ECOTROPIC (generous gift of Dr. Harvey Lodish) by the standard calcium phosphate precipitation technique. Plasmid pcB7-ECOTROPIC expresses the MCAT1 gene (ecotropic

15 retrovirus receptor cDNA) and hygromycin resistance gene transcribed from separate constitutive promoters. The transfected cells were selected for hygromycin resistance in 200 µg/ml hygromycin (Life Technologies). The stable, hygromycin-resistant clones were assayed for their ability to-take up and express reporter virus (MSCVPLAP). Fixation and staining for cell-bound alkaline phosphatase was performed by standard techniques. CHO clone 4B was chosen because

20 it transduced most efficiently at the highest dilution tested (i.e., 1:10,000), and was expanded for further analysis. Transduction of CHO4B with ecotropic retroviruses is equal to that achievable with NIH3T3 cells. Low passage number (passage 2-5), primary mouse neonatal cardiac endothelial cells (MNE) were prepared by standard techniques. MNE cells were cultured in a 1:1 vol./vol. admixture of EGM:EGM-2 (CLONETICS) in a 37 °C, humidified, 5.0% CO_2 incubator.

25 MNE cells were subcultured once weekly at a split ratio of approximately 1:3 in a 37 °C, humidified, 5.0% CO_2 incubator.

Northern blot analysis. Total RNA was prepared from confluent T-80 flasks of each of the transduced and untransduced cells using the QIAGEN RNAeasy kit with QIASHREDDER. 10 µg of total cellular RNA was denatured and resolved by electrophoresis in a 1.5% agarose gel,

30 and then blotted onto GENE-Screen+ (DuPont NEN) with 2X SSPE. The membrane was then UV cross-linked using a STRATAlinker. ^{32}P-radiolabeled cDNA probes were prepared from the

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fragments of DNA used for cloning the mouse 3-OST-1 and SEAP as described above.   -
Radiolabeled probes were prepared using 25ng of each template and the Amersham Megaprime
kit, and α ^{32}P dCTP from DuPont NEN according to the manufacturer's directions.
Hybridizations were performed in sealable plastic bags at 68 °C with 1 x 10^6 cpm of probe/ml in

5   10 ml of QUICKHYB (Stratagene, La Jolla, CA), following the manufacturer's instructions.
Post-hybridization washes were: once for 15 minutes in 1X SSPE, 1.0% SDS at 45 °C; and then
twice for 15 minutes each in 0.2X SSPE, 0.5% SDS 650C. After washing, the blots were briefly
air dried, placed in sealable plastic bags then exposed to Kodak XAR-MS film with intensifying
screens at -80 °C for from overnight to five days. Quantitation of hybridizing signal intensity was

10   performed using a Betascope 603 blot analyzer. Transcripts derived from the 5' LTR of these
engineered proviruses are large (ca. 7 kb). Since they are large, have multiple sites of
transcriptional initiation provirus (5' LTR and pgk promoters), and the 3-OST-1 construct has
more than one poly(A) addition signal, bona-fide hybridizable mRNA will appear as different sizes
in northern blot analysis. The total amount of hybridizing material detected, per sample lane, with

15   any one probe was used to calculate and compare mRNA expression levels.

Virion production. Virions were produced by programming ecotropic PHOENIX
packaging cells with recombinant provirus plasmids using the calcium phosphate transfection
technique. 10 µg/well of each recombinant retroviral construct plasmid was transfected via
calcium precipitation with an overnight incubation period. Following the precipitation step, the

20   cells were re-fed with 2 ml/well of fresh DMEM and incubated overnight. Each 2 ml of viral
supernatant was collected and flash-frozen in liquid nitrogen and stored at -80 °C, or used directly
after a low-speed centrifugation.

Transduction protocol. Target cells were trypsinized, counted with a Coulter cell counter
and then plated at 150,000 cells (NIH 3T3/CHO4B) or 50,000 cells (MNE) per well of a cluster-6

25   well plate. 24 hours later, target cells (<70% confluent) were incubated overnight with viral
supernatants containing as adjuvants either 5 µg/ml polybrene for NIH3T3/CHO4B or 25 µg/ml
DEAE-dextran (Pharmacia) for MNE. After 12 hours of virus exposure, the growth media was
replaced. CHO cells destined for FACS sorting were exposed to recombinant retrovirus two
times at a multiplicity of infection (MOI) of 0.3. MNE cells were transduced one time for 12

30   hours at an MOI of 0.74 for recombinant 3-OST-1 virus and 0.72 for recombinant SEAP virus.
Transduced cells were allowed to incubate in fresh growth medium for 48 hours prior to FACS to

allow for maximum proviral expression. Recombinant virus titers ranged from 1×10^5 -2×10^6 infectious particles per ml as measured with either NIH3T3 or CHO4B cells using FACS analysis scoring for EGFP positive cells. Virus titers were reduced approximately eight to ten-fold on primary MNE cells relative to NIH3T3.

5   Cell-Free Synthesis of Mouse and Human r3-OST-1.

Synthetic capped mouse and human 3-OST-1 mRNAs were generated from *Not*I linearized pNWS228 and *Hin*DIII linearized pNWS237, respectively, using T_3 polymerase and $m^7G(5')ppp(5')G$, as previously described (50). Unlabeled *in vitro* translation reactions (25 µl) contained 0.25 µg of synthetic mRNA, 1.8 µl canine pancreatic microsomal membranes

10   (Promega), 0.5 µl each of Amino Acid Mixture Minus Leucine and Amino Acid Mixture Minus Methionine, and were performed with nuclease-treated reticulocyte lysate (Promega), according to the manufacturer's specifications.

Measurement of HS^{act} Conversion Activity. The HS^{act} conversion activity, a 3-OST-1 catalyzed reaction which requires unlabeled PAPS to convert ^{35}S-HS^{inact} into ^{35}S-HS^{act}, of crude

15   and purified r3-OST-1 samples was determined by comparison against a standard curve generated with 1 to 32 units of previously purified native 3-OST-1, as previously described (26). The ^{35}S-HS^{inact} substrate was purified from metabolically labeled cell surface HS of exponentially growing clone 33 cells, as previously described (35).

Identification of Enzymatic Reaction Products

20   ^{35}S-labeling of HS by r3-OST-1. ^{35}S-labeled HS was generated by incubating the various forms of r3-OST-1 with $[^{35}S]PAPS$ and unlabeled HS^{inact}, which were prepared as previously described (26, 35). Wild-type and Protein A tagged r3-OST-1 (2500 units of HS^{act} conversion activity) purified from COS cell conditioned medium, were incubated in a 500 µl reaction mixture, as previously described (26), for 2 h at 37 °C and ^{35}S-labeled polysaccharides were purified by

25   DEAE-Sepharose chromatography as previously described (26). For cell-free synthesized r3-OST-1, ^{35}S-labeling of HS was performed in a reticulocyte lysate based reaction mixture (35) except that 100 µl reactions contained 100 to 300 units of *in vitro* translated r3-OST, 180 nM unlabeled HS^{inact}, 5 µM PAPS (60 x10^6 cpm) and samples were incubated at 37 °C for 2 h. The reaction was quenched by the addition of 300 µl of 267 mM NaCl, 13.3 µg/ml glycogen and

30   extraction against 600 µl of phenol/chloroform/isoamyl alcohol (25:24:1). ^{35}S-labeled GAGs were ethanol precipitated (35) and then isolated by DEAE chromatography as previously described (26).

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Identification of the Site of Sulfation on HS[act] and HS[inact]. The DEAE eluates containing
[35]S-labeled polysaccharide were vacuum concentrated to 1/5 volume, then desalted at a flow rate
of 0.9 ml/min on TSK G3000 PW$_{XL}$ (0.78 x 30 cm) and TSK G2500 PW$_{XL}$ (0.78 x 30 cm)
(TosoHaas) columns connected in series equilibrated in 0.1 M ammonium bicarbonate. The

5   desalted product was then affinity fractionated using AT/ConA gel to obtain HS[act] and HS[inact] as
described previously (26). Analysis of labeled products by treatment with GAG lyases and low
pH nitrous acid were performed as previously described (42). In addition, the HS[act] and HS[inact]
samples were each subjected to hydrazinolysis, high pH nitrous acid (pH 5.5), low pH nitrous acid
(pH 1.5), and sodium borohydride reduction with the resultant disaccharides characterized on

10 reverse phase ion pairing HPLC (RPIP-HPLC) as previously reported (33, 34). The identification
of [^{35}S]GlcA→AMN-3-O-SO$_3$ and [^{35}S]GlcA→AMN-3,6-O-(SO$_3$)$_2$ was confirmed by co-
chromatography on RPIC-HPLC with the appropriate ^{3}H-labeled disaccharide standards, as
described in prior publications (33,34).

Northern Blot Analysis

15   Total RNA from RFPEC and primary mouse CME cells was isolated by the method of
Chomczynski and Sacchi (51), whereas poly(A)+ RNA was isolated from HUVEC cells as
described above for LTA cells. Total RNA from the mast cell line CI.MC/C57.1 (C57.1) (52)
was from Dr. Stephen J. Galli (Beth Israel Hospital). Samples were resolved on 1.2%
formaldehyde-agarose gels and subjected to Northern blot analysis as previously described (50).

20 Mouse and human samples were hybridized with mouse or human probes, respectively, and
washed as described for library screening, above, except hybridizations were performed at 60 °C.

Peptide Sequencing and PCR Generation of a Mouse 3-O-Sulfotransferase-1 (3-OST-1) Probe

        The information necessary for the molecular cloning of mouse heparan sulfate D-
glucosaminyl 3-O-sulfotransferase-1 (3-OST-1) was obtained by sequencing the amino terminus

25 and Lys-C generated peptides of the enzyme that we had previously purified from large quantities
of serum-free tissue culture medium conditioned by an L cell line (26). These studies established
the structures of 14 partially overlapping peptides which encompass 185 amino acid residues.
Degenerate PCR primers were synthesized based on the sequence of the amino terminus (primer
1S) and two endopeptidase derived fragments (primers 2S, 2A, and 3A). When PCR was

30 performed on an LTA first strand cDNA template, products of about 210 (primers 1S/2A) and
780 (primers 1S/3A) and 610 (primers 2S/3A) bp were obtained, which suggests that all of the
primer sites are contained within a single cDNA. To confirm this supposition, the two largest

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fragments were cloned into pCR-Script Amp SK(+) and inserts were sequenced, which revealed that the 1S/3A product is 779 bp and contains the 611 bp 2S/3A product. The 779 bp insert encodes 12 of the sequenced peptide fragments and so was ^{32}P-labeled, as described above, and used as a probe for cDNA library screening.

5   Isolation and Characterization of Mouse 3-OST-1 cDNAs

An amplified λ Zap Express LTA cDNA library of 1.5×10^6 primary recombinants was constructed and 1.3×10^6 plaques were screened with the above described probe, which revealed 40 positives that were plaque purified and *in vivo* excised into plasmids. The cDNA inserts of each plasmid were characterized to eliminate duplicated recombinants due to library amplification.

10   Size was determined by liberating cDNA inserts with digestion at flanking *Eco*RI and *Xho*I restriction sites followed by agarose gel electrophoresis; furthermore, the sequence at both ends of each insert was obtained from flanking vector primer sites. This analysis revealed 25 unique primary recombinants which predominantly contained inserts of approximately 1.7, 2.3, or 3.3 kb. These different species were considered to reflect natural size variants of the mouse message since

15   northern blots of LTA poly(A)$^+$ RNA hybridized with 3-OST-1 probe revealed the same three size categories of message. The complete sequencing of 9 distinct primary recombinants, at least 2 from each size category, in conjunction with the partial sequencing of the remaining 16 clones showed that the size variants result from differences in the length of 5' untranslated region due to the insertion of 0-1629 bp at a single common internal point, the splice variant site. Most

20   importantly, all clones shared identical protein coding regions and, therefore, the characterization and analysis of only the shortest species, the Class 1 cDNA, which lacks additional sequence at the splice variant site, is described below.

Sequence data was obtained from 2 essentially full length Class 1 cDNAs, and 5 partial length cDNAs to create a composite cDNA structure of 1685 bp (SEQ ID NO: 1), excluding the

25   3' poly(A) tract. The 5' untranslated region is 322 bp with the splice variant site occurring between nucleotides 216 and 217. This region contains 6 ATG sites which do not conform to consensus initiation sites (53) and are followed by near in-frame termination codons. An open reading frame of 933 bp begins at position 323 with the first consensus initiation ATG (a purine occurs at -3) (53). The length of the 3' untranslated region from all of the cDNA clones analyzed

30   ranged from 301-430 bp. Within this terminal 129 bp, 5 distinct polyadenylation sites were observed and 13-18 bp upstream from each site is a variant of the consensus polyadenylation

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signal. Poly(A) tails were most frequently observed at the first site (position 1556, ~50% of clones).

Isolation and Characterization of Human 3-OST-1 cDNAs

Three clones containing partial length human 3-OST-1 cDNAs were identified by EST database searching (48) and were obtained from the TIGR/ATCC Special Collection, as described above. Sequencing of the insert ends revealed the clones to be essentially equivalent, as each contained the same 947 bp region of the human 3-OST-1 cDNA. The insert of I.M.A.G.E. Consortium CloneID 220372 was ^{32}P-labeled and used to screen 5 x 10^5 plaques from a λ TriplEx Brain cDNA library. Three positives were identified and isolated as TriplEx plasmids, and the largest cDNA 1.3 kb was sequenced completely.

The nucleic acid sequence of mouse and human 3-OST-1 cDNAs are ~85% identical. The largest isolated human clone contains 118 bp of 5' untranslated region with 2 nonconsensus ATG sites. The sequences of human and mouse cDNAs flanking the splice variant site on the 5' limit are distinct (positions 211-216 of SEQ ID NO: 1 and positions 5-10 of SEQ ID NO: 3), but on the 3' limit are identical (positions 217-222 of SEQ ID NO: 1 and positions 11-16 of SEQ ID NO: 3), which raises the possibility that human 3-OST-1 mRNA may also exhibit 5' splice variants. The first consensus ATG (with a purine occurring at -3 and a G at +4) (53) initiates an open reading frame of 921 bp. For all 4 human cDNA clones examined, only a single polyadenylation site was observed resulting in a 3' untranslated region of 266 bp, which is 26 bp less than the most frequently observed 3' limit for the mouse cDNAs.

Predicted Protein Structures of Mouse and Human 3-OST-1

The mouse and human cDNAs encode novel 311 and 307 amino acid proteins of 35,876 and 35,750 daltons, respectively, that exhibit 93% similarity. The deduced mouse primary structure contains regions corresponding to all 13 sequenced peptides and the amino terminus. For both types of 3-OST-1, the encoded protein is predicted to be an intraluminal resident. Kyte-Doolittle hydropathy analysis reveals only a single major hydrophobic region which begins at the amino terminus and lacks sufficient length for a membrane spanning domain. Moreover, the hydrophobic region differs from a membrane anchor in that it contains two glutamine residues and is not flanked by cationic residues. Thus, the above stretch of 18 residues constitutes a hydrophobic leader signal, and this region is followed by a signal peptidase cleavage site between amino acids 20 and 21, as determined by the method of von Heijne (54). The possibility of signal peptidase cleavage is supported by the amino-terminal analysis of mouse 3-OST-1, which began

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with His[21]. Given that heparan biosynthesis is considered to occur in the *trans*-Golgi, the above data suggest that the 3-OST-1 is an intraluminal enzyme. Just past the signal peptidase cleavage site, the mouse 3-OST-1 contains an extra 4 residues (Asp[24]-Pro[25]-Gly[26]-Pro[27]) not found in the human form. Both 3-OST-1 proteins exhibit 5 potential *N*-glycosylation sites which account for

5   the apparent discrepancy between the molecular weights of the predicted amino terminus trimmed enzyme (~34 kDa) and the previously purified enzyme (a broad band of 46 kDa was observed on SDS-PAGE) (26). Only two cysteine residues are present, and these closely spaced residues are likely to form a disulfide bond which generates a peptide loop of 10 amino acids. Interestingly, the carboxy 140 residue region is extremely basic (25% H, K, R; 12% E, D); however, this region

10   does not exhibit previously recognized heparin binding motifs.

Recombinant Expression of Mouse and Human 3-OST-1 Enzyme (r3-OST-1)

        Three distinct expression approaches were employed to confirm that the isolated cDNAs encode 3-OST-1 enzyme. The resulting recombinantly expressed 3-OST-1 enzyme was designated as r3-OST-1, to distinguish this form from the previously purified native 3-OST-1

15   enzyme. First, the vector pCMV-3-OST (a pcDNA3 derivative in which the CMV promoter transcribes the mouse 3-OST-1 cDNA) was transiently expressed in COS-7 cells and the resulting level of HS^{act} conversion activity accumulated in Serum-Free Medium over 32 h was measured, as described above. HS^{act} conversion activity is a 3-OST-1 catalyzed reaction which requires unlabeled PAPS to convert ^{35}S-HS^{inact} into ^{35}S-HS^{act}. Before or after pcDNA3 transfection,

20   typically COS-7 conditioned Serum-Free Medium contained a low but detectable amount of HS^{act} conversion activity, whereas transfection by pCMV-3-OST elevated levels ~2,000-fold.

        Second, to exclude the remote possibility that the expression of the mouse 3-OST-1 cDNA indirectly induces, rather than directly encodes, HS^{act} conversion activity, a Protein A/3-OST-1 fusion protein was analyzed. COS-7 cells were transiently transfected with pCMV-

25   ProA3-OST, a pCMV-3-OST derivative in which the amino-terminal 26 residues of the mouse 3-OST-1 are replaced with a Protein A tag, and Protein A tagged mouse r3-OST-1 was extracted with IgG agarose beads from 155 ml of conditioned Serum-Free Medium, as described above. The affinity purification recovered undetectable and less than 0.5% of initial HS^{act} conversion activity from control pcDNA3 and pCMV-3-OST transfection samples, respectively, whereas

30   ~7,000 units (10% recovery) were extracted from pCMV-ProA3-OST transfection samples. Thus, the mouse 3-OST-1 cDNA directly encodes HS^{act} conversion activity.

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Third, the activities of cell-free synthesized mouse and human r3-OST-1 were examined. Synthetic capped mouse and human 3-OST-1 mRNAs were generated by *in vitro* transcription and then *in vitro* translated with reticulocyte lysate in the presence and absence of canine pancreatic microsomal membranes, as described above. HS^{act} conversion activity was

5 undetectable in the control *in vitro* translation reactions which lacked mRNA template, with or without microsomal membranes. A low level HS^{act} conversion activity resulted from the addition of synthetic 3-OST-1 mRNA templates to translation reactions lacking microsomal membranes (mouse, 0.86 ± 0.028 units/μl, $n = 3$; human, 2.1 ± 0.063 units/μl, $n = 3$); however, ~15-fold greater levels occurred when microsomal membranes were included in translation reactions

10 (mouse, 14.3 ± 0.27 units/μl, $n = 3$; human, 32.4 ± 2.1 units/μl, $n = 3$). The apparent activation of nascent r3-OST-1 by co-translational processing within microsomes may result from signal peptidase cleavage, *N*-linked glycosylation, and/or a facilitation of correct protein folding. The slightly greater production from the human 3-OST-1 cDNA may reflect the more favorable context of the human initiation codon, or the reduced length of the human 5' untranslated region.

15 Independent of the above considerations, the above data confirm that isolated mouse and human cDNAs encode HS^{act} conversion activity.

Next, the biochemical specificity of the HS^{act} conversion activity generated from each expression approach was examined by incubating crude or purified enzyme with $[^{35}S]$PAPS and unlabeled HS^{inact}, recovering radiolabeled GAG by DEAE chromatography and characterizing the

20 resultant products. The HS^{act} conversion activity of the wild-type mouse r3-OST-1 produced by transfecting COS-7 cells with pCMV-3-OST (1.35×10^6 units in 240 ml of conditioned Serum-Free Medium) was first purified away from potential contaminating sulfotransferase activities by heparin-AF Toyopearl chromatography followed by 3',5'-ADP-agarose chromatography, which yielded ~1 μg of protein containing 340,000 units (~20,000-fold purification with 25% overall

25 recovery); whereas, the IgG agarose-purified Protein A tagged r3-OST-1 and *in vitro* translation reactions of mouse and human 3-OST-1 mRNA templates were directly analyzed, as described above. About $0.5 - 1 \times 10^6$ cpm of product was generated with purified wild-type r3-OST-1, purified Protein A tagged r3-OST-1, and nonpurified *in vitro* translation reactions containing mouse and human r3-OST-1, respectively. Portions of each labeled product were incubated with

30 purified heparitinase (0.5 units/ml) or chondroitinase ABC (0.5 units/ml) and HPLC-GPC analysis indicated that in all cases label was exclusively incorporated into HS. Portions of the labeled HS samples were also *N*-desulfated with nitrous acid at pH 1.5, and analyzed by P-2 polyacrylamide

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gel filtration to determine the amounts of liberated free [^{35}S]sulfate, as described above. The results demonstrated no increased generation of free [^{35}S]sulfate. Finally, portions of the labeled samples were AT affinity fractionated, which revealed that in each case ~40% of the ^{35}S-label was incorporated in HS^{act} and approximately ~60% of the ^{35}S-label was incorporated in HS^{inact}. The

5   labeled HS^{act} and HS^{inact} generated by the wild-type purified r3-OST-1 were chemically cleaved to disaccharides with nitrous acid treatment, appropriate ^{3}H-labeled disaccharides standards were added, and the ^{35}S- and ^{3}H-labeled species were coresolved by RPIP-HPLC as outlined above. The results show that the ^{35}S-label coelutes with [^{3}H]GlcA→AMN-3-O-SO$_3$ and [^{3}H]GlcA→AMN-3,6-O-(SO$_3$)$_2$, respectively. This approach also revealed that Protein A tagged

10 r3-OST-1, and *in vitro* translation derived mouse and human r3-OST-1 generated ^{35}S-HS which only contained ^{35}S-labeled disaccharides that coeluted with [^{3}H]GlcA→AMN-3-O-SO$_3$ and [^{3}H]GlcA→AMN-3,6-O-(SO$_3$)$_2$, respectively. It was previously shown that ^{35}S-labeled GlcA→AMN-3,6-O-(SO$_3$)$_2$ generated by purified 3-OST-1 enzyme contains ^{35}S solely in the 3-O-position (26). Thus, the expressed HS^{act} conversion activities exclusively catalyze the transfer of

15 sulfate to the 3-O- position of glucosamine units in HS^{act} and HS^{inact}.

Northern Analysis of Rodent and Human 3-OST-1 Expression

Northern blot analysis reveals the presence of 3-OST-1 message in different kinds of endothelial cells as well as a mast cell line. Both cell types have previously been shown to form HS^{act} and anticoagulant heparin, respectively (6, 8, 55). Three size categories of rodent 3-OST-1

20 mRNA (about 1.7, 2.3, 3.3 kb) and a single size species of the human message (about 1.7 kb) were evident. As described above, the mouse forms arise from differential splicing within the 5' untranslated region. Similar size categories are also expressed by rat (RFPEC) endothelial cells, suggesting a similar mechanism of origin. The abundance of each category varies with each cell line, which suggests that a mechanism exists to regulate such differential splicing. The

25 immortalized mouse mast cell line, C57.1, expresses high levels of the same three size categories, which suggests that expression of a single 3-OST-1 gene is required for the synthesis of both HS^{act} and anticoagulant heparin.

The 3-OST-1 Sequence Defines a Heparan Sulfotransferase Family

Extensive computer-aided data bank searching revealed the 3-OST-1 protein to be a

30 previously unidentified protein; furthermore, the carboxy-terminal 250 residues exhibit a low homology (~30% similarity) to many previously identified sulfotransferases (which are typically

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~300 residues in length) including chondroitin-, aryl-/phenol-, *N*-hydroxyarylamine-, alcohol-/hydroxysteroid-, flavonol-, and nodulation factor sulfotransferases. We also observed a slightly greater homology (~40% similarity) to a functionally unidentified open reading frame of 247 amino acids from Aeromonas salmonicida (GenBank accession number L37077). More

5   importantly, the 3-OST-1 protein exhibits ~50% similarity with all previously identified forms of the heparan biosynthetic enzyme *N*-deacetylase/*N*-sulfotransferase (NST). In particular, extensive homology exists across the entire 250-270 carboxy-terminal residues of these enzymes. Thus, it appears that a common sulfotransferase structure is shared by two distinct types of heparan biosynthetic enzyme. Given that NST is a bifunctional enzyme, the above observation suggests

10   that NST enzymes possess sulfotransferase activity within a ~270 residue carboxy-terminal domain, whereas deacetylase activity would be contained within the remaining ~560 luminal residues. Interestingly, the region of consensus Lys^{302}-Arg^{323}, which encompasses the presumptive cysteine bridged peptide loop (described above), exhibits complete conservation for 12 of the 22 residues (including both cysteines) among all 3-OST-1 and NST species.

15   Identification and molecular cloning of 3-OST-2, 3-OST-3A, 3-OST-3B and 3-OST-4

The 3-OST-1 protein exhibits a COOH-terminal region of ~260 residues which was determined to be a sulfotransferase (ST) domain based on homology to all known sulfotransferases. The National Center for Biotechnology Information data bank of expressed sequence tags (ESTs) was searched with amino acid sequences of the ST domain from the human

20   3-OST-1 cDNA to reveal seven human cDNAs encoding three novel related species. The forms were subsequently designated as 3-OST-2 (I.M.A.G.E. Consortium (LLNL) CloneID c-20d10), 3-OST-3 (Clone ID 284542) and 3-OST-4 (Clone IDs HIBCX69, IB727, 166466, 23279, and c-3ie01). These EST clones were obtained from the TIGR/ATCC Special Collection, and the inserts were completely sequenced, revealing that all clones were of partial length.

25   To obtain full length clones, isoform specific probes were generated from the EST clones and used to screen λ TriplEx human cDNA libraries. 7 and 4 additional 3-OST-2 and 3-OST-4 cDNAs were isolated from a brain library, and 8 new 3-OST-3 cDNAs were recovered from a liver library. The cDNA inserts were completely sequenced, revealing the full length form for 3-OST-2 as well as 2 distinct full length forms for 3-OST-3 (3-OST-3A and 3-OST-3B). The

30   additional 3-OST-4 clones were also of partial length.

3-OST-2, 3-OST-3A, 3-OST-3B and 3-OST-4 Protein Structures and Activities

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The 3-OST-2, 3-OST-3A, and 3-OST-3B proteins are 367, 406, and 390 amino acids in length, respectively. All three proteins conform to the architecture of a type-II integral membrane protein. These proteins and the partial length 3-OST-4 share a common (85% similarity) ST domain region of ~260 amino acid at their COOH-terminus. To characterize the encoded HS

5   sulfotransferase activities, the 3-OST-2, 3-OST-3A, and 3-OST-3B cDNAs were individually expressed in COS-7 cells.

The analysis of transfected cell extracts demonstrated that each enzyme transfers sulfate specifically to the 3-O position of glucosamine residues within HS; however distinct specificities occur. 3-OST-2 preferentially sulfates regions containing GlcA 2S→GlcNS to generate

10 GlcA 2S→GlcNS 3S; whereas both 3-OST-3A, and 3-OST-3B recognize regions with IdoA 2S→GlcNS to generate IdoA 2S→GlcNS 3S.

Expression Patterns Indicate Biological Function

The biologic function of these novel enzymes was elucidated by performing northern blot analysis. 3-OST-4 is exclusively expressed in the brain, whereas 3-OST-2 mRNA predominantly

15 occurs in the brain with minor levels also found in heart, lung, skeletal muscle and placenta. 3-OST-3 forms occur in virtually all tissues but with barely detectable levels in brain, low levels in heart, lung, skeletal muscle and kidney, and extremely abundant expression in liver and placenta. Thus 3-OST-2 and 3-OST-4 appear to be the brain counterparts of 3-OST-3. The product of 3-OST-3 (IdoA 2S→GlcNS 3S) has previously been shown to be extremely abundant in HSPGs

20 isolated from the glomerular basement membrane (GBM) of the kidney. These HSPGs are critical to regulating the permselectivity of the GBM. This function occurs through interactions with extracellular matrix components that regulate the pore size of the matrix. Given that the liver, placenta, and kidney glomerulus are all responsible for the filtration of macromolecular components from blood and all exhibit high 3-OST-3 expression, it appears that 3-OST-3 serves a

25 common function in each situation: to regulate macromolecular permeability. In this functional regard, the high brain expression of 3-OST-2 and 3-OST-4 correlates with the major molecular permeability barrier of the central nervous system, the blood brain barrier.

Therapeutic Utilities

The 3-OST heparan biosynthetic enzymes may be generated by recombinant expression of

30 the isolated cDNAs to generate novel glycosaminoglycan drugs of specific structure through an *in vitro* biochemical synthesis approach. Specifically, 3-OST-1 may be used to generate

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anticoagulant pentasaccharides, which may be administered subcutaneously to treat thrombotic

disorders such as deep vein thrombosis and pulmonary embolism. The 3-OST-1 enzyme may also

be used to generate an orally absorbable form of pentasaccharide from an appropriate

carbohydrate substrate linked to a hydrophobic group. In an analogous fashion, specific

5   glycosaminoglycan products may be generated from 3-OST-2, 3-OST-3 and 3-OST-4, which may

be used as therapeutics to alter macromolecular permeability of various vascular beds. Drugs

which reduce capillary permeability may, at the very least, be used to treat (1) microproteinurea

and macroproteinurea of renal diseases including diabetic nephropathy and the various forms of

glomerulonephrititis; (2) neoplastic growths by limiting nutrient supply to tumors; and (3)

10   inflammatory diseases were macromolecular constituents of the plasma are required for initiating

and maintaining a localized inflammation. Conversely, drugs which enhance capillary permeability

may be used (1) as an adjunctive treatment to facilitate pharmacological access to vascular beds,

which exhibit highly selective drug entry, such as the blood brain barrier and the placental barrier;

and (2) to enhance nutrient supply to under-perfused tissues such as the myocardium after an

15   infarct.

Specific heparan sulfate structures regulate additional biologic processes by interacting

with numerous protein effector molecules including growth and differentiation factors (e.g., FGF

family members, HB-EGF, HGF/SF, interferon γ, PDGF, SDGF, and VEGF/VPF), chemokines

(e.g., MIP-1β, RANTES, and GRO), receptors (e.g., TGF-β receptors), mast cell proteases,

20   protease inhibitors (e.g., AT, heparin cofactor II, leuserpin, plasminogen activator inhibitor-1,

protease nexins), degradative enzymes (e.g., elastase, acetylcholinesterase, extracellular

superoxide dismutase, thrombin, tissue plasminogen activator, lipoprotein lipase, hepatic and

pancreatic triglyceride lipase, and cholesterol esterase), apolipoproteins (e.g., apoB and apoE),

matrix components (e.g., fibronectin, wnt-1, interstitial collagens, laminin, pleiotropin, tenascin,

25   thrombospondin, and vitronectin) viral coat proteins (e.g., gC and gB of HSV types I and II, gC-

II of CMV, and gp120 of HIV), nuclear proteins (e.g., c-fos, c-jun, RNA and DNA polymerases,

and steroid receptors), cellular adhesion molecules (e.g., L-selectin, P-selectin, PECAM-1, and N-

CAM) and other molecules (e.g., HB-GAM/pleiothrophin, amphoterin, and PF4).

Using routine methods (e.g., site-directed mutagenesis) the available 3-OST cDNAs may

30   be selectively mutated to alter substrate recognition properties so as to produce enzymes that

generate novel glycosaminoglycan structures which modulate the biologic processes regulated by

the above effector molecules. Thus, novel drugs may also be biochemically synthesized from

recombinantly expressed mutated enzymes. Such substances may serve to (1) enhance growth or regeneration of specific cell types such as the endothelial cells of the heart after infarction, or neurons in neurodegenerative diseases; (2) suppress undesirable cell growth in conditions such as cancer (either directly by acting on the cancers cells or indirectly by preventing endothelial cells from neovascularizing the tumor), atherosclerosis (by preventing smooth muscle cell growth), and inflammatory diseases characterized by cellular proliferation; (3) prevent metastasis of tumors by modulating cell/matrix interactions; (4) reduce the destructive side effects of inflammatory reactions by inhibiting degradative enzymes or by activating inhibitory molecules (e.g. protease inhibitors) which may be directly or indirectly protective by limiting extravasation of lymphocytes; (5) modulate serum lipid levels by enhancing or reducing the cellular or tissue uptake or degradation of specific lipoprotein classes; (6) treat viral infections by preventing viral entry into cells; and (7) facilitate axon regeneration subsequent to nerve severing.

Bacterial expression of 3-OST-1. The human and mouse 3-OST-1 proteins have been expressed as active, soluble protein in *E. coli*. This has been achieved using the pET system from NOVAGEN (Madison, WI). The human and mouse 3-OST-1 cDNA's were PCR amplified with pfu DNA polymerase and purified cloned plasmids as template. The primers that were used were designed to amplify a cDNA fragment starting, in frame, after the native signal sequence and including the native translational termination codon. Additionally, the PCR primers were designed to include restriction sites that would facilitate cloning into the vectors described below in the correct transcriptional/translational reading frames. 3-OST-1 was cloned into vectors pET12a, 15B and 28a according to the manufacturer's instructions. This places the 3-OST-1 cDNA downstream of a powerful, inducible T7 transcription site and includes an efficient Shine-Dalgarno sequence at the appropriate distance from the initiator methionine of the construct.

Good yields of active protein result from IPTG induction at room temperature. The specific activity appears to be less than purified, or Baculovirus/sf9 produced material. The exact magnitude of the diminution of activity is unclear at this time; however, it may be 10-1000 fold. The presently preferred purification scheme is: (1) Induction at 22 °C. (2) Sonication of bacteria, centrifugation to remove inclusion bodies and cell debris, purification of crude bacterial sonicate on heparin sepharose as described elsewhere. (3) PAP column chromatography. (4) Gel permeation chromatography. Step (4) is only needed for obtaining monomeric, pure 3-OST-1, and not for active protein preparation.

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CLAIMS

What is claimed is:

1   1.   An isolated nucleic acid encoding at least a functional fragment of a 3-OST protein.

1   2.   An isolated nucleic acid as in claim 1 wherein said nucleic acid encodes a 3-OST protein

2   comprising a mature 3-OST-1 protein selected from the group consisting of mature murine 3-

3   OST-1 and mature human 3-OST-1.

1   3.   An isolated nucleic acid as in claim 1 wherein said nucleic acid encodes a 3-OST protein

2   comprising a protein selected from the group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-

3   OST-3B, 3-OST-4, and ce3-OST.

1   4.   An isolated nucleic acid as in claim 1 wherein said nucleic acid encodes a 3-O-

2   sulfotransferase domain of a 3-OST protein selected from the group consisting of 3-OST-1, 3-

3   OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, ce3-OST.

1   5.   An isolated nucleic acid as in claim 1 wherein said nucleic acid comprises a nucleotide

2   sequence selected from nucleotide sequences within:

3   (a) SEQ ID NO: 1;

4   (b) SEQ ID NO: 3;

5   (c) SEQ ID NO: 5;

6   (d) SEQ ID NO: 7;

7   (e) SEQ ID NO: 9;

8   (f) SEQ ID NO: 11;

9   (g) a sequence having at least 60% nucleotide sequence identity with at least one of

10   (a)-(f) and encoding a functional fragment having sequence-specific HS binding affinity or 3-O-

11   sulfotransferase activity; and

12   (h) a sequence differing from a sequence of (a)-(g) only by the substitution of

13   synonymous codons.

1   6.   An isolated nucleic acid as in claim 1 wherein said nucleic acid comprises a nucleotide

2   sequence encoding a polypeptide selected from the group consisting of:

3   (a) residues 21-52, 260-269, 250-276, 53-311, or 21-307 of SEQ ID NO: 2;

4   (b) residues 21-48, 256-265, 246-272, 49-307, or 21-303 of SEQ ID NO: 4;

5   (c) residues 42-109, 313-325, 303-332, or 110-367 of SEQ ID NO: 6;

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6   (d) residues 44-147, 351-363, 341-370, or 148-406 of SEQ ID NO: 8;

7   (e) residues 66-132, 336-348, 326-355, or 133-390 of SEQ ID NO: 10;

8   (f) residues 396-408, 386-4150, or 207-456 of SEQ ID NO: 12;

9   (g) residues 240-250, 230-257, 23-291 of SEQ ID NO: 15;

10   (h) a sequence having at least 60% amino acid sequence similarity with at least one of

11   (a)-(g) and encoding a functional fragment having sequence-specific HS binding affinity or 3-O-

12   sulfotransferase activity; and

13   (i) a sequence comprising a chimera of at least two of sequences (a)-(h).

1   7.   An isolated nucleic acid comprising at least 16 consecutive nucleotides of a nucleotide

2   sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5,

3   SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11.

1   8.   A host cell transformed with a nucleic acid of any one of claims 1-7, or a descendant

2   thereof.

1   9.   A host cell as in claim 8 wherein said host cell is selected from the group consisting of

2   bacterial cells, yeast cells, and insect cells.

1   10.   A host cell as in claim 8 wherein said host cell is selected from the group consisting of

2   somatic cells, fetal cells, embryonic stem cells, zygotes, gametes, germ line cells, and transgenic

3   animal cells.

1   11.   A host cell as in claim 8 wherein said cell is a mammalian cell.

1   12.   A host cell as in claim 11 wherein said cell is selected from the group consisting of. COS-7

2   cells, CHO, murine primary cardiac microvascular endothelial cells (CME), murine mast cell line

3   C57.1, human primary endothelial cells of umbilical vein (HUVEC), F9 embryonal carcinoma

4   cells, rat fat pad endothelial cells (RFPEC), L cells, and cells derived from the transgenic animals

5   of the invention.

1   13.   A substantially pure protein preparation comprising at least a functional fragment of a 3-

2   OST protein.

1   14.   A substantially pure protein preparation as in claim 13 wherein said 3-OST protein is

2   selected from the group consisting of mature murine 3-OST-1 and mature human 3-OST-1.

1   15.   A substantially pure protein as in claim 13 wherein said 3-OST protein is selected from the

2   group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST.

1   16.   A substantially pure protein preparation as in claim 13 wherein said functional fragment

2   comprises a 3-O-sulfotransferase domain of a 3-OST protein selected from the group consisting

3   of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST.

1   17.   A substantially pure protein preparation as in claim 13 wherein said functional fragment

2   comprises an amino acid sequence selected from amino acid sequences within:

3   (a) SEQ ID NO: 2;

4   (b) SEQ ID NO: 4;

5   (c) SEQ ID NO: 6;

6   (d) SEQ ID NO: 8;

7   (e) SEQ ID NO: 10;

8   (f) SEQ ID NO: 12;

9   (g) SEQ ID NO: 15;

10   (h) a sequence having at least 60% amino acid similarity with at least one of (a)-(g) and

11   having sequence-specific HS binding affinity or 3-O-sulfotransferase activity; and

12   (i) a sequence comprising a chimera of at least two of sequences (a)-(h).

1   18.   A substantially pure protein preparation as in claim 13 wherein said functional fragment

2   comprises an amino acid sequence selected from the group consisting of:

3   (a) residues 21-52, 260-269, 250-276, 53-311, or 21-307 of SEQ ID NO: 2;

4   (b) residues 21-48, 256-265, 246-272, 49-307, or 21-303 of SEQ ID NO: 4;

5   (c) residues 42-109, 313-325, 303-332, or 110-367 of SEQ ID NO: 6;

6   (d) residues 44-147, 351-363, 341-370, or 148-406 of SEQ ID NO: 8;

7   (e) residues 66-132, 336-348, 326-355, or 133-390 of SEQ ID NO: 10;

8   (f) residues 396-408, 386-415, or 207-456 of SEQ ID NO: 12;

9   (g) residues 240-250, 230-257, 23-291 of SEQ ID NO: 15;

10   (h) a sequence having at least 60% amino acid sequence similarity with at least one of

11   (a)-(g) and encoding a functional fragment having sequence-specific HS binding affinity or 3-O-

12   sulfotransferase activity; and

13   (i) a sequence comprising a chimera of at least two of sequences (a)-(h).

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1   19.   A method of 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan

2   or proteoglycan polysaccharides comprising:

3   contacting said preparation with at least a 3-O-sulfotransferase domain of a 3-OST protein

4   in the presence of a sulfate donor under conditions which permit sulfation of said residues;

5   wherein, said 3-OST protein is selected from the group consisting of 3-OST-1, 3-OST-2,

6   3-OST-3A, 3-OST-3B, 3-OST-4, ce3-OST, and conservative substitution variants or chimeras

7   thereof.

1   20.   A method of 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan

2   or proteoglycan polysaccharides, wherein said polysaccharides include a polysaccharide sequence

3   of GlcA→GlcNS ±6S comprising:

4   contacting said preparation with a 3-OST-1 protein in the presence of a sulfate donor

5   under conditions which permit said 3-OST-1 to convert said GlcA→GlcNS ±6S sequence to

6   GlcA→GlcNS 3S ±6S.

7   wherein the 3-OST-1 protein is selected from the group consisting of murine 3-OST-1,

8   human 3-OST-1, mature murine 3-OST-1, mature human 3-OST-1, a functional fragment of a 3-

9   OST-1 having 3-O-sulfotransferase activity, a conservative substitution variant of 3-OST-1

10 having 3-O-sulfotransferase activity, and a chimeric 3-OST-1 having 3-O-sulfotransferase activity.

1   21.   A method as in claim 20, wherein said GlcA→GlcNS ±6S polysaccharide sequence

2   comprises a part of a polysaccharide sequence selected from the group consisting of:

3   (a) GlcA→GlcNS ±6S→IdoA 2S→ GlcNS ±6S;

4   (b) IdoA→GlcNAc 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;

5   (c) IdoA→GlcNS 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;

6   (d) IdoA→GlcNAc→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;

7   (e) IdoA→GlcNS→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;

8   (f) IdoA→GlcNAc 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS;

9   (g) IdoA→GlcNS 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS;

1   22.   A method of 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan

2   or proteoglycan polysaccharides, wherein said polysaccharides include a polysaccharide sequence

3   of GlcA 2S→GlcNS comprising:

4   contacting said preparation with a 3-OST-2 protein in the presence of a sulfate donor

5   under conditions which permit said 3-OST-2 to convert said GlcA 2S→GlcNS sequence to GlcA

6   2S→GlcNS 3S.

7   wherein the 3-OST-2 protein is selected from the group consisting of 3-OST-2, a

8   functional fragment of a 3-OST-2 having 3-O-sulfotransferase activity, a conservative substitution

9   variant of 3-OST-2 having 3-O-sulfotransferase activity, and a chimeric 3-OST-2 having 3-O-

10   sulfotransferase activity.

1   23.   A method as in claim 22, wherein said GlcA 2S→GlcNS polysaccharide sequence

2   comprises a part of a GlcNS→GlcA 2S→GlcNS polysaccharide sequence.

1   24.   A method of 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan

2   or proteoglycan polysaccharides, wherein said polysaccharides include a polysaccharide sequence

3   of IdoA 2S→GlcNS comprising:

4   contacting said preparation with a 3-OST-3 protein in the presence of a sulfate donor

5   under conditions which permit said 3-OST-3 to convert said IdoA 2S→GlcNS sequence to IdoA

6   2S→GlcNS 3S.

7   wherein the 3-OST-3 protein is selected from the group consisting of 3-OST-3A, 3-OST-

8   3B, a functional fragment of a 3-OST-3 having 3-O-sulfotransferase activity, a conservative

9   substitution variant of 3-OST-3 having 3-O-sulfotransferase activity, and a chimeric 3-OST-3

10   having 3-O-sulfotransferase activity.

1   25.   A method as in claim 24, wherein said IdoA 2S→GlcNS polysaccharide sequence

2   comprises a part of a GlcNS→IdoA 2S→GlcNS polysaccharide sequence.

1   26.   A method for enriching the AT-binding fraction in a preparation of heparan sulfates,

2   wherein said preparation includes a polysaccharide sequence of GlcA→GlcNS ±6S comprising:

3   contacting said preparation with 3-OST-1 protein in the presence of a sulfate donor under

4   conditions which permit said 3-OST-1 to convert said GlcA→GlcNS ±6S sequence to

5   GlcA→GlcNS 3S ±6S, thereby increasing the fraction of AT-binding heparan sulfates.

1   27.   A method for converting HS^{act} precursor to HS^{act} in a preparation of heparan sulfates,

2   wherein said preparation includes HS^{act} precursor polysaccharides including a sequence of

3   GlcA→GlcNS ±6S comprising:

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4   contacting said preparation with 3-OST-1 protein in the presence of a sulfate donor under

5   conditions which permit said 3-OST-1 to convert said GlcA→GlcNS ±6S sequence to

6   GlcA→GlcNS 3S ±6S, thereby converting HS^{act} precursor to HS^{act}.

1   28.   A method as in any one of claims 19-28 wherein said sulfate donor is PAPS.

1   29.   A non-human animal model, wherein a genome of said animal, or an ancestor thereof,

2   wherein said recombinant construct has introduced a modification into said genome, said

3   modification selected from the group consisting of insertion of a nucleic acid encoding at least a

4   functional fragment of a conspecific wild type 3-OST protein, insertion of a nucleic acid encoding

5   at least a functional fragment of a transpecific allelic variant of the 3-OST sequences, insertion of

6   nucleic acid encoding at least a functional fragment of an allelic variant of 3-OST sequence,

7   inactivation of an endogenous 3-OST gene, and insertion by homologous recombination of a

8   reporter gene coupled to 3-OST transcriptional elements.

1   30.   An animal as in claim 29 wherein said modification is insertion of nucleic acid encoding at

2   least a functional fragment of wild type 3-OST selecting from the sequence consisting of the

3   SPLAG-domain, the cysteine-binding peptide loop, and the ~260 residue ST domain.

1   31.   An animal as in claim 29 wherein said animal is selected from the group consisting of rats,

2   mice, hamsters, guinea pigs, rabbit, dogs, cats, goats, sheep, pigs, and non-human primates.

1   32.   An animal as in claim 29 wherein said animal is an invertebrate.

1   33.   A method of producing antibodies which selectively bind to a 3-OST protein comprising

2   the steps of

3   administering an immunogenically effective amount of a 3-OST epitope to an animal;

4   allowing said animal to produce antibodies to said epitope; and

5   obtaining said antibodies from said animal or from a cell culture derived therefrom.

1   34.   A substantially pure preparation of antibody which selectively binds to an epitope of a 3-

2   OST protein.

1   35.   A substantially pure preparation of an antibody as claimed in 34 wherein said antibody

2   selectively binds to at least a fragment of 3-OST.

1   36.   A cell line producing an antibody of any one of the claims 34.

1   37.   A method for identifying compounds which can modulate the expression of a 3-OST gene

2   comprising steps of

3   providing a cell expressing a nucleic acid under the control of a 3-OST regulatory

4   sequence;

5   contacting said cell with at least one candidate compound; and

6   assaying for a change in the in the expression of said nucleic acid.

1   38.   The method of claim 37, wherein said nucleic acid comprises a marker gene and a 3-OST

2   gene

1   39.   The method of claim 37, wherein said assaying step comprises detecting a change in 3-

2   OST mRNA level.

1   40.   The method of claim 37, wherein said assaying step comprises detecting a change in 3-

2   OST protein encoded by said nucleic acid.

1   41.   A method of determining partial sequence information for complex polysaccharides

2   comprising the steps of:

3   contacting a first sample of polysaccharide with at least one ligand which binds

4   polysaccharides in a sequence specific manner;

5   contacting the resulting polysaccharide-ligand complex with at least one agent that

6   modifies complex polysaccharides;

7   contacting a second sample of polysaccharide with the same modifying agent;

8   comparing said first and second samples for ligand-specific inhibition of modifications

9   caused by said modifying agent.

1   42.   The method of claim 41, wherein said complex polysaccharide is a glycosaminoglycan.

1   43.   The method of claim 41, wherein said ligand is catalytically inactive.

1   44.   The method of claim 41, wherein said ligand is an inactive 3-OST.

1   45.   The method of claim 41, wherein said agent that modifies polysaccharides is selected from

2   the group consisting of epimerases, lyases, sulfotransferases, N-acetyltransferases, N-

3   deacetylases, epimerases.

1   46.   The method of claim 45, wherein said modifying agent is a sequence specific degrading

2   agent.

1   47.   The method of claim 45, wherein said modifying agent is a non-sequence specific

2   degrading agent.

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1   48.   The method of claims 46, wherein said degrading agent is a lyase.

1   49.   The method of claim 47, wherein said non-sequence specific degrading agent nitrous acid.

1   50.   The method of claim 45, further comprising affinity purifying said modified first and
2   second samples.

1   51.   The method of claim 45, wherein the step of comparing includes a comparison of size
2   profiles.

1   52.   A method of determining partial sequence information for complex polysaccharides
2   comprising the steps of:
3   contacting a first sample of complex polysaccharides with a 3-OST protein in the presence
4   of a sulfate donor under conditions which permit sulfation by said 3-OST;
5   contacting said first sample and a second sample with at least one enzyme which cleaves
6   polysaccharides in a sequence-specific manner;
7   determining the size profiles of the resulting fragments.

1   53.   The method of claim 52, wherein the determining the size profile step further comprises
2   the step of comparing said first sample to a second sample cleaved by the same enzymes.

1   54.   The method of claim 52, wherein said enzymes which degrade polysaccharides in a
2   sequence specific manner are selected from the group consisting of polysaccharide lyases,
3   heparinase I, heparinase II, and heparinase III

1   55.   A method of determining partial sequence information for a sample containing complex
2   polysaccharides comprising the steps of:
3   contacting said sample of polysaccharide with a 3-OST protein which lacks enzymatic
4   function with a under conditions which permit said 3-OST protein to bind to said polysaccharide
5   in a sequence specific manner;
6   applying said sample to an affinity column;
7   applying degrading agents to said column;
8   analyzing the resulting degradation products.

1   56.   The method of claim 55, further comprising repeating the steps applying degrading agents
2   and analyzing using a series of different sequence specific polysaccharide cleavage enzymes.
3

1   57.   An isolated nucleic acid comprising a 5' untranslated regulatory region of a 3-OST gene
2   operably joined to a marker gene.

1   58.   A host cell transformed with the isolated nucleic acid of claim 57, or a descendent thereof.

1   59.   A method of identifying compounds capable of modulating the expression of a 3-OST

2   gene comprising contacting a candidate compound with the transformed host cell of claim 58 and

3   assaying for changes in expression of said marker.

1   60.   A method as in claim 59, wherein said regulatory region comprise the 5' untranslated

2   region of SEQ ID NO: 16.

```
m3-OST-1   MTLLLLGAVL LVAQPQLVHS HPAAPGPGLK QQELLRKVII   40
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m3-OST-1   LPEDTGEGTA SNGSTQQLPQ TIIIGVRKGG TRALLEMLSL   80
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m3-OST-1   HPDVAAAENE VHFFDWEEHY SQGLGWYLTQ MPFSSPHQLT   120
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h3-OST-1   HPDVAAAENE VHFFDWEEHY SHGLGWYLSQ MPFSWPHQLT   116

m3-OST-1   VEKTPAYFTS PKVPERIHSM NPTIRLLLIL RDPSERVLSD   160
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h3-OST-1   VEKTPAYFTS PKVPERVYSM NPSIRLLLIL RDPSERVLSD   156

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m3-OST-1   LSPQINASNF YFNKTKGFYC LRDSGKDRCL HESKGRAHPQ   280
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m3-OST-1   VDPKLLDKLH EYFHEPNKKF FKLVGRTFDW H   311
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h3-OST-1   VDPKLLNKLH EYFHEPNKKF FELVGRTFDW H   307
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Figure 1

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23   TSKKRFPDAIIVGVKKSGTRALLEFLRVNPLI    KAPGPEVHFFDKNFN KGLEWYREQMPETKFGEV     TIEKS    ce3-OST
193  GEKK LPQALIIGVKKGGTRALLEAIRVHPDV    RAVGVEPHFFDRN  YEKGLEWYRNVMPKTLD    GQITMEKT    3-OST-4
148  GS  KQLPQAIIIGVKKGGTRALLEFLRVHPDV   RAVGAEPHFFDRS  YDKGLAWYRDLMPRTLD    GQITMEKT    3-OST-3A
110  GT  KRLPQALIVGVKKGGTRAVLEFIRVHPDV   RALGTEPHFFDRN  YGRGLDWYRSLMPRTLE    SQITLEKT    3-OST-2
49   GSAQQLPQTIIIGVRKGGTRALLEMLSLHPDV   AAAENEVHFFDWEEHYSHGLGWYLSQMPFSWP     HQLTVEKT    3-OST-1


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93   PAYFHSKMAPERIKSLNPNTKIIIVVRDPVTRAISDYTQSSSKRKRVGLM PSFETMAVGNCANWLRTNCTTKTRGVNAG    ce3-OST
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217  PSYFVTREAPARISAMSKDTKLIVVVRDPVTRAISDYTQT       LSKRPDIPTFESLTFKNRT      AGLIDTS    3-OST-3A
179  PSYFVTQEAPRRIFNMSRDTKLIVVVRDPVTRAISDYTQT       LSKKPDIPTFEGLSFRNRT      LGLVDVS    3-OST-2
121  PAYFTSPKVPERVYSMNPSIRLLILRDPSERVLSDYTQVFYNHMQKHKPYPSIEEFLVRD           GRLNVD    3-OST-1


749  NRCLVPGWYATHIERWLSAYHANQIILVLDGKLLRTEPAKVMDMVQKFLGVTNTIDYHKTLAFDPKKGFWC   QLLEGGKT   NST-1
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172  WGAIRIGVYHKMKRWLDHFPIENIHIVDGEKLISNPADEISATEKFLGLKPVAK PEKFGVDPIKKFPCIK   NEDGKL    ce3-OST
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283  WSAIQIGIYAKHLEHWLRHFPIRQMLFVSGERLISDPAGELGRVQDFLGLKRFIT  DKHFYFNKTKGFPCLKKAEGSSRP    3-OST-3A
245  WNAIRIGMYVLHLESWLQYFPLAQIHFVSGERLITDPAGEMGRVQDFLGIKRFIT  DKHFYFNKTKGFPCLKKTESSLLP    3-OST-2
188  YKALNRSLYHVHMQNWLRFFPLRHIHIVDGDRLIRDPFPEIQKVERFLKSPQIN   ASNFYFNKTKGFYCLR   DSGRD    3-OST-1


827  RCLGKSKGRKYPEMDLDSRAFLKDYYRDHNIELSKLLYKMGQTLPTWLREDLQNTR    NST-1
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407  RCLGKSKGRTHPRIDPDVIHRLRKFYKPFNLMFYQMTGQDFQWEQEEGDK          3-OST-4
362  HCLGKTKGRTHPEIDREVVRRLREFYRP FNLKFYQMTGHDFGWDG              3-OST-3A
324  RCLGKSKGRTHVQIDPEVIDQLREFYRPYNIKFYETVGQDFRWE                3-OST-2
264  RCLHESKGRAHPQVDPKLLNKLHEYFHEPNKKFFELVGRTFDWH                3-OST-1
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Figure 2
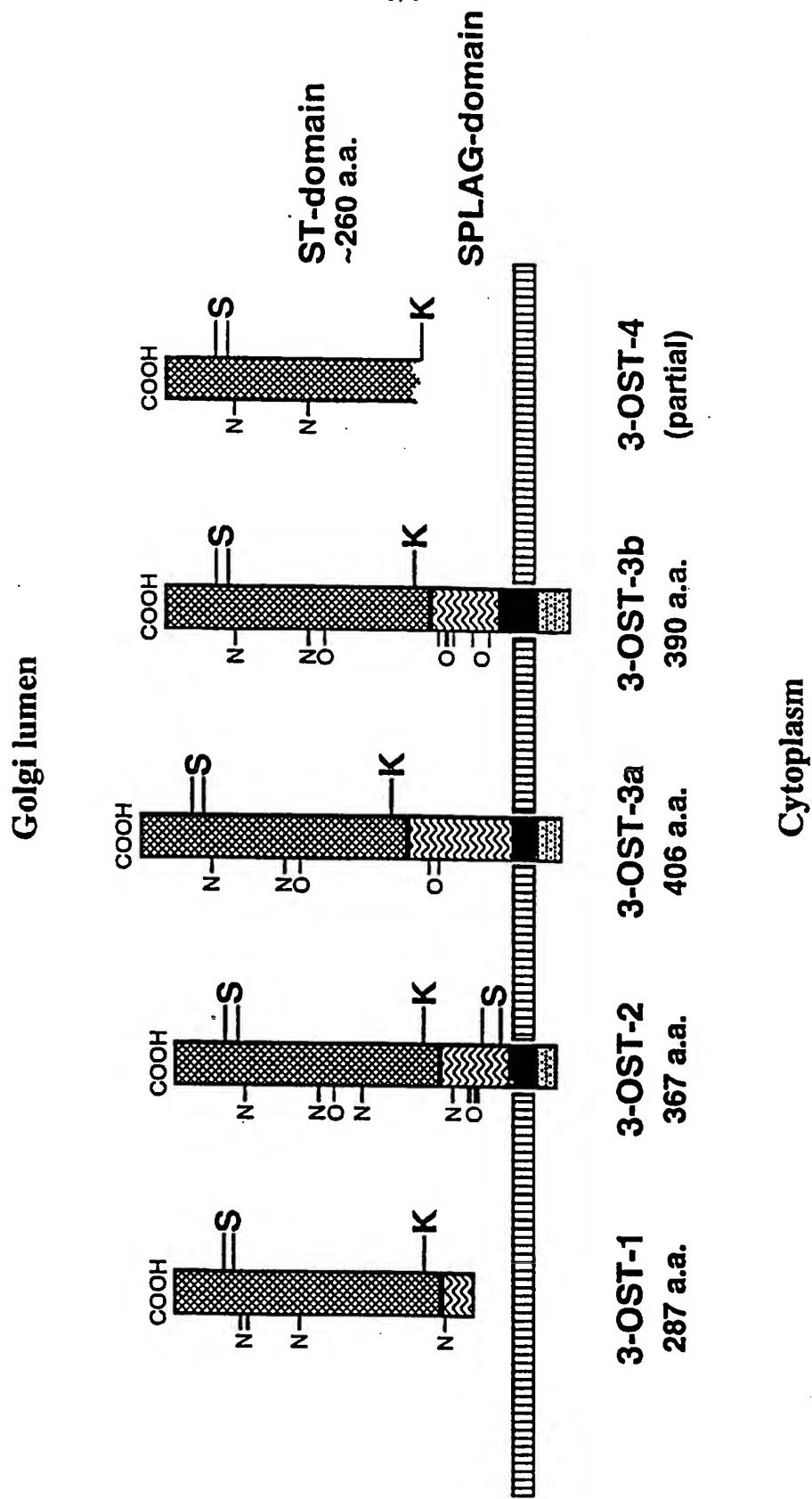
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<110> ROSENBERG, Robert D
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      FRITZE, Linda M. S.
      SCHWARTZ, John J
      ZHANG, Lijuan
      Massachusetts Institute of Technology

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Leu Glu Met Leu Ser Leu His Pro Asp Val Ala Ala Ala Glu Asn Glu
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gtc cat ttc ttt gac tgg gag gag cat tac agc caa ggc ctg ggc tgg    640
·Val His Phe Phe Asp Trp Glu Glu His Tyr Ser Gln Gly Leu Gly Trp
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Tyr Leu Thr Gln Met Pro Phe Ser Ser Pro His Gln Leu Thr Val Glu
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Lys Thr Pro Ala Tyr Phe Thr Ser Pro Lys Val Pro Glu Arg Ile His
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Val Glu Arg Phe Leu Lys Leu Ser Pro Gln Ile Asn Ala Ser Asn Phe
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<213> Mus musculus

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Met Thr Leu Leu Leu Leu Gly Ala Val Leu Leu Val Ala Gln Pro Gln
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Leu Val His Ser His Pro Ala Ala Pro Gly Pro Gly Leu Lys Gln Gln
            20   25   30

Glu Leu Leu Arg Lys Val Ile Ile Leu Pro Glu Asp Thr Gly Glu Gly
        35   40   45

Thr Ala Ser Asn Gly Ser Thr Gln Gln Leu Pro Gln Thr Ile Ile Ile
        50   55   60

Gly Val Arg Lys Gly Gly Thr Arg Ala Leu Leu Glu Met Leu Ser Leu
65   70   75   80

His Pro Asp Val Ala Ala Ala Glu Asn Glu Val His Phe Phe Asp Trp
                85   90   95

Glu Glu His Tyr Ser Gln Gly Leu Gly Trp Tyr Leu Thr Gln Met Pro
            100   105   110

Phe Ser Ser Pro His Gln Leu Thr Val Glu Lys Thr Pro Ala Tyr Phe
            115   120   125

Thr Ser Pro Lys Val Pro Glu Arg Ile His Ser Met Asn Pro Thr Ile
        130   135   140

Arg Leu Leu Leu Ile Leu Arg Asp Pro Ser Glu Arg Val Leu Ser Asp
145   150   155   160

Tyr Thr Gln Val Leu Tyr Asn His Leu Gln Lys His Lys Pro Tyr Pro
                165   170   175

Pro Ile Glu Asp Leu Leu Met Arg Asp Gly Arg Leu Asn Leu Asp Tyr
            180   185   190

Lys Ala Leu Asn Arg Ser Leu Tyr His Ala His Met Leu Asn Trp Leu
        195   200   205

Arg Phe Phe Pro Leu Gly His Ile His Ile Val Asp Gly Asp Arg Leu
    210   215   220

Ile Arg Asp Pro Phe Pro Glu Ile Gln Lys Val Glu Arg Phe Leu Lys
225   230   235   240

Leu Ser Pro Gln Ile Asn Ala Ser Asn Phe Tyr Phe Asn Lys Thr Lys
                245   250   255

Gly Phe Tyr Cys Leu Arg Asp Ser Gly Lys Asp Arg Cys Leu His Glu
            260   265   270

Ser Lys Gly Arg Ala His Pro Gln Val Asp Pro Lys Leu Leu Asp Lys
        275   280   285

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Leu His Glu Tyr Phe His Glu Pro Asn Lys Lys Phe Phe Lys Leu Val
    290             295             300

Gly Arg Thr Phe Asp Trp His
305             310


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ctgcggcact tcatgaccca gcggcgcgcg gcccagtgaa gccaccgtgg tgtccagc    118

atg gcc gcg ctg ctc ctg ggc gcg gtg ctg ctg gtg gcc cag ccc cag  166
Met Ala Ala Leu Leu Leu Gly Ala Val Leu Leu Val Ala Gln Pro Gln
 1               5                  10                  15

cta gtg cct tcc cgc ccc gcc gag cta ggc cag cag gag ctt ctg cgg  214
Leu Val Pro Ser Arg Pro Ala Glu Leu Gly Gln Gln Glu Leu Leu Arg
             20                  25                  30

aaa gcg ggg acc ctc cag gat gac gtc cgc gat ggc gtg gcc cca aac  262
Lys Ala Gly Thr Leu Gln Asp Asp Val Arg Asp Gly Val Ala Pro Asn
         35                  40                  45

ggc tct gcc cag cag ttg ccg cag acc atc atc atc ggc gtg cgc aag  310
Gly Ser Ala Gln Gln Leu Pro Gln Thr Ile Ile Ile Gly Val Arg Lys
     50                  55                  60

ggc ggc acg cgc gca ctg ctg gag atg ctc agc ctg cac ccc gac gtg  358
Gly Gly Thr Arg Ala Leu Leu Glu Met Leu Ser Leu His Pro Asp Val
65                  70                  75                  80

gcg gcc gcg gag aac gag gtc cac ttc ttc gac tgg gag gag cat tac  406
Ala Ala Ala Glu Asn Glu Val His Phe Phe Asp Trp Glu Glu His Tyr
                 85                  90                  95

agc cac ggc ttg ggc tgg tac ctc agc cag atg ccc ttc tcc tgg cca  454
Ser His Gly Leu Gly Trp Tyr Leu Ser Gln Met Pro Phe Ser Trp Pro
             100                 105                 110

cac cag ctc aca gtg gag aag acc ccc gcg tat ttc acg tcg ccc aaa  502
His Gln Leu Thr Val Glu Lys Thr Pro Ala Tyr Phe Thr Ser Pro Lys
             115                 120                 125

gtg cct gag cga gtc tac agc atg aac ccg tcc atc cgg ctg ctg ctc  550
Val Pro Glu Arg Val Tyr Ser Met Asn Pro Ser Ile Arg Leu Leu Leu
     130                 135                 140

atc ctg cga gac ccg tcg gag cgc gtg cta tct gac tac acc caa gtg  598
Ile Leu Arg Asp Pro Ser Glu Arg Val Leu Ser Asp Tyr Thr Gln Val
145                 150                 155                 160

ttc tac aac cac atg cag aag cac aag ccc tac ccg tcc atc gag gag  646
Phe Tyr Asn His Met Gln Lys His Lys Pro Tyr Pro Ser Ile Glu Glu
                 165                 170                 175

ttc ctg gtg cgc gat ggc agg ctc aat gtg gac tac aag gcc ctc aac  694
Phe Leu Val Arg Asp Gly Arg Leu Asn Val Asp Tyr Lys Ala Leu Asn
```

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                   180                    185                      190

cgc agc ctc tac cac gtg cac atg cag aac tgg ctg cgc ttt ttc ccg    742
Arg Ser Leu Tyr His Val His Met Gln Asn Trp Leu Arg Phe Phe Pro
        195                 200                 205

ctg cgc cac atc cac att gtg gac ggc gac cgc ctc atc agg gac ccc    790
Leu Arg His Ile His Ile Val Asp Gly Asp Arg Leu Ile Arg Asp Pro
        210                 215                 220

ttc cct gag atc caa aag gtc gag agg ttc cta aag ctg tcg ccg cag    838
Phe Pro Glu Ile Gln Lys Val Glu Arg Phe Leu Lys Leu Ser Pro Gln
225                 230                 235                 240

atc aat gct tcg aac ttc tac ttt aac aaa acc aag ggc ttt tac tgc    886
Ile Asn Ala Ser Asn Phe Tyr Phe Asn Lys Thr Lys Gly Phe Tyr Cys
                245                 250                 255

ctg cgg gac agc ggc cgg gac cgc tgc tta cat gag tcc aaa ggc cgg    934
Leu Arg Asp Ser Gly Arg Asp Arg Cys Leu His Glu Ser Lys Gly Arg
            260                 265                 270

gcg cac ccc caa gtc gat ccc aaa cta ctc aat aaa ctg cac gaa tat    982
Ala His Pro Gln Val Asp Pro Lys Leu Leu Asn Lys Leu His Glu Tyr
            275                 280                 285

ttt cat gag cca aat aag aag ttc ttc gag ctt gtt ggc aga aca ttt    1030
Phe His Glu Pro Asn Lys Lys Phe Phe Glu Leu Val Gly Arg Thr Phe
        290                 295                 300

gac tgg cac tgatttgcaa taagctaagc tcagaaactt tcctactgta           1079
Asp Trp His
305

agttctggtg tacatctgag gggaaaaaga attttaaaaa agcatttaag gtataatttta 1139

tttgtaaaat ccataaagta cttctgtaca gtattagatt cacaattgcc atatatacta 1199

gttatatttt tctacttgtt aaatggaggg cattttgtat tgttttтcat ggttgttaac 1259

attgtgtaat atgtctctat atgaaggaac taaactattt cactga               1305
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<211> 307
<212> PRT
<213> Homo sapiens

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Met Ala Ala Leu Leu Leu Gly Ala Val Leu Leu Val Ala Gln Pro Gln
 1               5                  10                  15

Leu Val Pro Ser Arg Pro Ala Glu Leu Gly Gln Gln Glu Leu Leu Arg
            20                  25                  30

Lys Ala Gly Thr Leu Gln Asp Asp Val Arg Asp Gly Val Ala Pro Asn
        35                  40                  45

Gly Ser Ala Gln Gln Leu Pro Gln Thr Ile Ile Ile Gly Val Arg Lys
        50                  55                  60

Gly Gly Thr Arg Ala Leu Leu Glu Met Leu Ser Leu His Pro Asp Val
65                  70                  75                  80

Ala Ala Ala Glu Asn Glu Val His Phe Phe Asp Trp Glu Glu His Tyr
                85                  90                  95

Ser His Gly Leu Gly Trp Tyr Leu S r Gln Met Pro Phe Ser Trp Pro
```

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            100                  105                  110

His Gln Leu Thr Val Glu Lys Thr Pro Ala Tyr Phe Thr Ser Pro Lys
        115                  120                  125

Val Pro Glu Arg Val Tyr Ser Met Asn Pro Ser Ile Arg Leu Leu Leu
    130                  135                  140

Ile Leu Arg Asp Pro Ser Glu Arg Val Leu Ser Asp Tyr Thr Gln Val
145                  150                  155                  160

Phe Tyr Asn His Met Gln Lys His Lys Pro Tyr Pro Ser Ile Glu Glu
                165                  170                  175

Phe Leu Val Arg Asp Gly Arg Leu Asn Val Asp Tyr Lys Ala Leu Asn
            180                  185                  190

Arg Ser Leu Tyr His Val His Met Gln Asn Trp Leu Arg Phe Phe Pro
        195                  200                  205

Leu Arg His Ile His Ile Val Asp Gly Asp Arg Leu Ile Arg Asp Pro
    210                  215                  220

Phe Pro Glu Ile Gln Lys Val Glu Arg Phe Leu Lys Leu Ser Pro Gln
225                  230                  235                  240

Ile Asn Ala Ser Asn Phe Tyr Phe Asn Lys Thr Lys Gly Phe Tyr Cys
                245                  250                  255

Leu Arg Asp Ser Gly Arg Asp Arg Cys Leu His Glu Ser Lys Gly Arg
            260                  265                  270

Ala His Pro Gln Val Asp Pro Lys Leu Leu Asn Lys Leu His Glu Tyr
        275                  280                  285

Phe His Glu Pro Asn Lys Lys Phe Phe Glu Leu Val Gly Arg Thr Phe
    290                  295                  300

Asp Trp His His
305


<210> 5
<211> 1951
<212> DNA
<213> Homo sapiens

<220>
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<222> (73)..(1173)
<223> human 3-OST-2

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gcccatggag cc atg gcc tat agg gtc ctg ggc cgc gcg ggg cca cct cag 111
              Met Ala Tyr Arg Val Leu Gly Arg Ala Gly Pro Pro Gln
              1                5                  10

ccg cgg agg gcg cgc agg ctg ctc ttc gcc ttc acg ctc tcg ctc tcc  159
Pro Arg Arg Ala Arg Arg Leu Leu Phe Ala Phe Thr Leu Ser Leu Ser
    15                  20                  25

tgc act tac ctg tgt tac agc ttc ctg tgc tgc tgc gac gac ctg ggt  207
Cys Thr Tyr Leu Cys Tyr Ser Phe Leu Cys Cys Cys Asp Asp Leu Gly
30                  35                  40                  45

cgg agc cgc ctc ctc ggc gcg cct cgc tgc ctc cgc ggc ccc agc gcg  255
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Arg Ser Arg Leu Leu Gly Ala Pro Arg Cys Leu Arg Gly Pro Ser Ala
            50   55   60

ggc ggc cag aaa ctt ctc cag aag tcc cgc ccc tgt gat ccc tcc ggg   303
Gly Gly Gln Lys L u Leu Gln Lys Ser Arg Pro Cys Asp Pro Ser Gly
            65   70   75

ccg acg ccc agc gag ccc agc gct ccc agc gcg ccc gcc gcc gcc gtg   351
Pro Thr Pro Ser Glu Pro Ser Ala Pro Ser Ala Pro Ala Ala Ala Val
        80   85   90

ccc gcc cct cgc ctc tcc ggt tcc aac cac tcc ggc tca ccc aag ctg   399
Pro Ala Pro Arg Leu Ser Gly Ser Asn His Ser Gly Ser Pro Lys Leu
        95   100   105

ggt acc aag cgg ttg ccc caa gcc ctc att gtg ggc gtg aag aag ggg   447
Gly Thr Lys Arg Leu Pro Gln Ala Leu Ile Val Gly Val Lys Lys Gly
.110   115   120   125

ggc acc cgg gcc gtg ctg gag ttt atc cga gta cac ccg gac gtg cgg   495
Gly Thr Arg Ala Val Leu Glu Phe Ile Arg Val His Pro Asp Val Arg
                130   135   140

gcc ttg ggc acg gaa ccc cac ttc ttt gac agg aac tac ggc cgc ggg   543
Ala Leu Gly Thr Glu Pro His Phe Phe Asp Arg Asn Tyr Gly Arg Gly
            145   150   155

ctg gat tgg tac agg agc ctg atg ccc agg acc ctc gag agc cag atc   591
Leu Asp Trp Tyr Arg Ser Leu Met Pro Arg Thr Leu Glu Ser Gln Ile
            160   165   170

acg ctg gag aag acg ccc agc tac ttt gtc act caa gag gct cct cga   639
Thr Leu Glu Lys Thr Pro Ser Tyr Phe Val Thr Gln Glu Ala Pro Arg
        175   180   185

cgc atc ttc aac atg tcc cga gac acc aag ctg atc gtg gtt gtg cgg   687
Arg Ile Phe Asn Met Ser Arg Asp Thr Lys Leu Ile Val Val Val Arg
190   195   200   205

aac cct gtg acc cgt gcc atc tct gat tac acg cag aca ctc tcc aag   735
Asn Pro Val Thr Arg Ala Ile Ser Asp Tyr Thr Gln Thr Leu Ser Lys
                210   215   220

aag ccc gac atc ccg acc ttt gag ggc ctc tcc ttc cgc aac cgc acc   783
Lys Pro Asp Ile Pro Thr Phe Glu Gly Leu Ser Phe Arg Asn Arg Thr
                225   230   235

ctg ggc ctg gtg gac gtg tcg tgg aac gcc atc cgc atc ggc atg tac   831
Leu Gly Leu Val Asp Val Ser Trp Asn Ala Ile Arg Ile Gly Met Tyr
            240   245   250

gtg ctg cac ctg gag agc tgg ctg cag tac ttc ccg cta gct cag att   879
Val Leu His Leu Glu Ser Trp Leu Gln Tyr Phe Pro Leu Ala Gln Ile
        255   260   265

cac ttc gtc agt ggc gag cga ctc atc act gac ccg gcc ggc gag atg   927
His Phe Val Ser Gly Glu Arg Leu Ile Thr Asp Pro Ala Gly Glu Met
270   275   280   285

ggg cga gtc cag gac ttc ctg ggc att aag aga ttc atc acg gac aag   975
Gly Arg Val Gln Asp Phe Leu Gly Ile Lys Arg Phe Ile Thr Asp Lys
                290   295   300

cac ttc tat ttc aac aag acc aaa gga ttc cct tgc ttg aaa aaa aca   1023
His Phe Tyr Phe Asn Lys Thr Lys Gly Phe Pro Cys Leu Lys Lys Thr
            305   310   315

gaa tcg agc ctc ctg cct cga tgc ttg ggc aaa tca aaa ggg aga act   1071

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        Glu Ser Ser Leu Leu Pro Arg Cys Leu Gly Lys Ser Lys Gly Arg Thr
            320             325             330

        cat gta cag att gat cct gaa gtg ata gac cag ctc cga gaa ttt tat   1119
        His Val Gln Ile Asp Pro Glu Val Ile Asp Gln Leu Arg Glu Phe Tyr
            335             340             345

        aga ccg tat aat atc aaa ttt tat gaa acc gtt ggg cag gac ttc agg   1167
        Arg Pro Tyr Asn Ile Lys Phe Tyr Glu Thr Val Gly Gln Asp Phe Arg
        350             355             360             365

        tgg gaa taagcccacg aaaggaaagg gctctcaagg gctcttctgc tcatctcttc    1223
        Trp Glu

        cgtgagattt gctcccagac cctcttatct ccctccaaca aaccctggct ccagccccct 1283

        ttcccaactt gagttgcatc atcttggaac caggaagccc agctaaagcc aagagaccag 1343

        agagtctctg ccactagttt tcatcagtct gttcaagcaa agttgatctg ctcctggcac 1403

        gtccagtaaa ttccagaatc attctccttt ctgcccataa agggccttgg agaattgctt 1463

        taagaagagt gaatgttcca atgatgatag atattataag cgacgatggt tctgttgcta 1523

        tgaacacagc agtcggtccc tgtcattgtc cacccaggag tggccttgtt aattccaagt 1583

        ggcatgtatc ttccctctga gcttcatttc ttcaagatgc tctgggtggt gggatgggag 1643

        accatcctca gccctcctca gaccttatca attcattgag agattgcaaa gctgaaagca 1703

        cctccggcca ctcctgggag acagaccctt tggtgatgaa ataaaccagt gacttcagag 1763

        cctatggtct caactgtgct tgaaaaacac tgtctctgaa acaactttg tgattctccc 1823

        tgctccctgt ggacaaaagc acataattct gctgttacgg gtactttgct catacgagct 1883

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        tgtcctta                                                          1951
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<212> PRT
<213> Homo sapiens

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Met Ala Tyr Arg Val Leu Gly Arg Ala Gly Pro Pro Gln Pro Arg Arg
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Ala Arg Arg Leu Leu Phe Ala Phe Thr Leu Ser Leu Ser Cys Thr Tyr
            20              25              30

Leu Cys Tyr Ser Phe Leu Cys Cys Cys Asp Asp Leu Gly Arg Ser Arg
            35              40              45

Leu Leu Gly Ala Pro Arg Cys Leu Arg Gly Pro Ser Ala Gly Gly Gln
        50              55              60

Lys Leu Leu Gln Lys Ser Arg Pro Cys Asp Pro Ser Gly Pro Thr Pro
65              70              75              80

Ser Glu Pro Ser Ala Pro Ser Ala Pro Ala Ala Val Pro Ala Pro
            85              90              95

Arg Leu Ser Gly Ser Asn His Ser Gly Ser Pro Lys Leu Gly Thr Lys
            100             105             110
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Arg Leu Pro Gln Ala Leu Ile Val Gly Val Lys Lys Gly Gly Thr Arg
        115             120             125

Ala Val Leu Glu Phe Ile Arg Val His Pro Asp Val Arg Ala Leu Gly
    130             135             140

Thr Glu Pro His Phe Phe Asp Arg Asn Tyr Gly Arg Gly Leu Asp Trp
145             150             155             160

Tyr Arg Ser Leu Met Pro Arg Thr Leu Glu Ser Gln Ile Thr Leu Glu
                165             170             175

Lys Thr Pro Ser Tyr Phe Val Thr Gln Glu Ala Pro Arg Arg Ile Phe
            180             185             190

Asn Met Ser Arg Asp Thr Lys Leu Ile Val Val Val Arg Asn Pro Val
        195             200             205

Thr Arg Ala Ile Ser Asp Tyr Thr Gln Thr Leu Ser Lys Lys Pro Asp
    210             215             220

Ile Pro Thr Phe Glu Gly Leu Ser Phe Arg Asn Arg Thr Leu Gly Leu
225             230             235             240

Val Asp Val Ser Trp Asn Ala Ile Arg Ile Gly Met Tyr Val Leu His
                245             250             255

Leu Glu Ser Trp Leu Gln Tyr Phe Pro Leu Ala Gln Ile His Phe Val
                260             265             270

Ser Gly Glu Arg Leu Ile Thr Asp Pro Ala Gly Glu Met Gly Arg Val
            275             280             285

Gln Asp Phe Leu Gly Ile Lys Arg Phe Ile Thr Asp Lys His Phe Tyr
    290             295             300

Phe Asn Lys Thr Lys Gly Phe Pro Cys Leu Lys Lys Thr Glu Ser Ser
305             310             315             320

Leu Leu Pro Arg Cys Leu Gly Lys Ser Lys Gly Arg Thr His Val Gln
            325             330             335

Ile Asp Pro Glu Val Ile Asp Gln Leu Arg Glu Phe Tyr Arg Pro Tyr
        340             345             350

Asn Ile Lys Phe Tyr Glu Thr Val Gly Gln Asp Phe Arg Trp Glu
        355             360             365
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<210> 7
<211> 2314
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (799)..(2016)
<223> human 3-OST-3A

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caggtcccgc gcagcccagc ccagcccagc cacgcggctc acaggtgggg tccaagagca   120

gtttggagca acccggcgct acggagaggg gtggacggct ctgcacgggc ctcctgtctc   180

ccgctcgggc agagggactc gggggaccct cgctccttgg ccgagagaac ctgaactcgg   240
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gcggagagaa cgcgcccagg cgggcaaggg gaccagagaa agccgggggct ggaagtcact  300

gtcgctcgcc actgtctgga gcgcacggag cgcagaggcc cggcagccgc gcgtgccctc  360

ccggggaccg agccagtgat gcaggatcgc tgagcggaga tccgcgccga gaagtctctc  420

ggggccgggg ctgagacgca cgccttcgac accgctgcca agaccccgat tccggcgact  480

cttgcgggga accgaggggc caaggctgcc ccaagctcag gacttgggcg agtctaagac  540

gatggtttct taagcacgga cccgcgttcc ccttcccgcc ccctcgactg gaggcaggga  600

tcctgcgcgg ggccccgggg attccgtttc cccgcggagc cccggccgct gcctcccggg  660

acagttcgca cggccacagg ggcgcacggc gatgtggcct ccgtccagcg cgctggcccg  720

ccggggggat gctctggcac ctgtcggggt ccaggcctag catggccggc gcgttgcccg  780
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acgtcgcctc cggctagg atg gcc cct ccg ggc ccg gcc agt gcc ctc tcc     831
                     Met Ala Pro Pro Gly Pro Ala Ser Ala Leu Ser
                      1               5                   10

acc tcg gcc gag ccg ctg tcc cgc agc atc ttc cgg aag ttc ttg ctg     879
Thr Ser Ala Glu Pro Leu Ser Arg Ser Ile Phe Arg Lys Phe Leu Leu
            15                  20                  25

atg ctc tgc tcc ctg ctc acg tcc ctt tac gtc ttc tac tgc ctg gcc     927
Met Leu Cys Ser Leu Leu Thr Ser Leu Tyr Val Phe Tyr Cys Leu Ala
        30                  35                  40

gag cgc tgc cag acc ctg tcc ggc ccc gtc gtg ggg ctg tcc ggc ggc     975
Glu Arg Cys Gln Thr Leu Ser Gly Pro Val Val Gly Leu Ser Gly Gly
    45                  50                  55

ggc gag gag gcg ggg gcc cct ggt ggc ggc gtc ctg gcc gga ggc ccg    1023
Gly Glu Glu Ala Gly Ala Pro Gly Gly Gly Val Leu Ala Gly Gly Pro
60                  65                  70                  75

agg gag ctg gcg gtg tgg ccg gcg gcg gca cag aga aag cgc ctc ctg    1071
Arg Glu Leu Ala Val Trp Pro Ala Ala Ala Gln Arg Lys Arg Leu Leu
                80                  85                  90

caa ctg ccg cag tgg cgg agg cgc cgg ccg ccc gcg ccc cgc gac gac    1119
Gln Leu Pro Gln Trp Arg Arg Arg Arg Pro Pro Ala Pro Arg Asp Asp
                95                  100                 105

ggc gag gag gcg gcc tgg gaa gaa gag tcc cct ggc ctg tca ggg ggt    1167
Gly Glu Glu Ala Ala Trp Glu Glu Glu Ser Pro Gly Leu Ser Gly Gly
            110                 115                 120

ccg ggc ggc tcc ggg gcc gga agc acc gtg gcc gag gcc ccg ccg ggg    1215
Pro Gly Gly Ser Gly Ala Gly Ser Thr Val Ala Glu Ala Pro Pro Gly
        125                 130                 135

acc ctg gcg ctg ctc ctg gac gaa ggc agc aag cag ctg ccg cag gcc    1263
Thr Leu Ala Leu Leu Leu Asp Glu Gly Ser Lys Gln Leu Pro Gln Ala
140                 145                 150                 155

atc atc atc gga gtg aag aag ggc ggc acg cgg gcg ctg ctg gag ttc    1311
Ile Ile Ile Gly Val Lys Lys Gly Gly Thr Arg Ala Leu Leu Glu Phe
                160                 165                 170

ctg cgc gtg cac ccc gac gtg cgc gcc gtg ggc gcc gag ccc cac ttc    1359
Leu Arg Val His Pro Asp Val Arg Ala Val Gly Ala Glu Pro His Phe
                175                 180                 185

ttc gac cgc agc tac gac aag ggc ctc gcc tgg tac cgg gac ctg atg    1407
Phe Asp Arg Ser Tyr Asp Lys Gly Leu Ala Trp Tyr Arg Asp Leu M t
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11/25

ccc aga acc ctg gac ggg cag atc acc atg gag aag acg ccc agt tac   1455
Pro Arg Thr Leu Asp Gly Gln Ile Thr Met Glu Lys Thr Pro Ser Tyr
    205   210   215

ttc gtc acg cgg gag gcc ccc gcg cgc atc tcg gcc atg tcc aag gac   1503
Phe Val Thr Arg Glu Ala Pro Ala Arg Ile Ser Ala Met Ser Lys Asp
220   225   230   235

acc aag ctc atc gtg gtg gtg cgg gac ccg gtg acc agg gcc atc tcg   1551
Thr Lys Leu Ile Val Val Val Arg Asp Pro Val Thr Arg Ala Ile Ser
               240   245   250

gac tac acg cag acg ctg tcc aag cgg ccc gac atc ccc acc ttc gag   1599
Asp Tyr Thr Gln Thr Leu Ser Lys Arg Pro Asp Ile Pro Thr Phe Glu
               255   260   265

agc ttg acg ttc aaa aac agg aca gcg ggc ctc atc gac acg tcg tgg   1647
Ser Leu Thr Phe Lys Asn Arg Thr Ala Gly Leu Ile Asp Thr Ser Trp
               270   275   280

agc gcc atc cag atc ggc atc tac gcc aag cac ctg gag cac tgg ctg   1695
Ser Ala Ile Gln Ile Gly Ile Tyr Ala Lys His Leu Glu His Trp Leu
    285   290   295

cgc cac ttc ccc atc cgc cag atg ctc ttc gtg agc ggc gag cgg ctc   1743
Arg His Phe Pro Ile Arg Gln Met Leu Phe Val Ser Gly Glu Arg Leu
300   305   310   315

atc agc gac ccg gcc ggg gag ctg ggc cgc gtg caa gac ttc ctg ggc   1791
Ile Ser Asp Pro Ala Gly Glu Leu Gly Arg Val Gln Asp Phe Leu Gly
               320   325   330

ctc aag agg atc atc acg gac aag cac ttc tac ttc aac aag acc aag   1839
Leu Lys Arg Ile Ile Thr Asp Lys His Phe Tyr Phe Asn Lys Thr Lys
               335   340   345

ggc ttc ccc tgc ctg aag aag gcg gag ggc agc agc cgg ccc cat tgc   1887
Gly Phe Pro Cys Leu Lys Lys Ala Glu Gly Ser Ser Arg Pro His Cys
    350   355   360

ctg ggc aag acc aag ggc agg acc cat cct gag atc gac cgc gag gtg   1935
Leu Gly Lys Thr Lys Gly Arg Thr His Pro Glu Ile Asp Arg Glu Val
    365   370   375

gtg cgc agg ctg cgc gag ttc tac cgg cct ttc aac ctc aag ttc tac   1983
Val Arg Arg Leu Arg Glu Phe Tyr Arg Pro Phe Asn Leu Lys Phe Tyr
380   385   390   395

cag atg acc ggg cac gac ttt ggc tgg gat gga taaccatata atttaaaaag   2036
Gln Met Thr Gly His Asp Phe Gly Trp Asp Gly
               400   405

aaaaaaaaaa tcaaaatata atatatttt ttaccaatcg gtagagaaga gacagtttaa   2096

tatttgtgct gaaaatatgt ttcagtattt ttttcaatga atgttaagag attgttctca   2156

ctcccgcccc atcttaatgt ataaccaaca ccaaacacgt ggatcaacag aaaaggaaaa   2216

tttcactcgt ctaaacactt tcaatttttca gtttttattt tatgttctat atacccagtc   2276

ataaagtata agcatcagtt gtcattaaaa gttttcag   2314

<210> 8
<211> 406
<212> PRT

<213> Homo sapiens

<400> 8

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Met Ala Pro Pro Gly Pro Ala Ser Ala Leu Ser Thr Ser Ala Glu Pro
1               5                   10                  15

Leu Ser Arg Ser Ile Phe Arg Lys Phe Leu Leu Met Leu Cys Ser Leu
            20                  25                  30

Leu Thr Ser Leu Tyr Val Phe Tyr Cys Leu Ala Glu Arg Cys Gln Thr
        35              40                  45

Leu Ser Gly Pro Val Val Gly Leu Ser Gly Gly Glu Glu Ala Gly
    50                  55                  60

Ala Pro Gly Gly Gly Val Leu Ala Gly Gly Pro Arg Glu Leu Ala Val
65                  70                  75                  80

Trp Pro Ala Ala Ala Gln Arg Lys Arg Leu Leu Gln Leu Pro Gln Trp
                85                  90                  95

Arg Arg Arg Arg Pro Pro Ala Pro Arg Asp Asp Gly Glu Glu Ala Ala
            100                 105                 110

Trp Glu Glu Glu Ser Pro Gly Leu Ser Gly Gly Pro Gly Gly Ser Gly
        115                 120                 125

Ala Gly Ser Thr Val Ala Glu Ala Pro Pro Gly Thr Leu Ala Leu Leu
    130                 135                 140

Leu Asp Glu Gly Ser Lys Gln Leu Pro Gln Ala Ile Ile Ile Gly Val
145                 150                 155                 160

Lys Lys Gly Gly Thr Arg Ala Leu Leu Glu Phe Leu Arg Val His Pro
                165                 170                 175

Asp Val Arg Ala Val Gly Ala Glu Pro His Phe Phe Asp Arg Ser Tyr
            180                 185                 190

Asp Lys Gly Leu Ala Trp Tyr Arg Asp Leu Met Pro Arg Thr Leu Asp
            195                 200                 205

Gly Gln Ile Thr Met Glu Lys Thr Pro Ser Tyr Phe Val Thr Arg Glu
    210                 215                 220

Ala Pro Ala Arg Ile Ser Ala Met Ser Lys Asp Thr Lys Leu Ile Val
225                 230                 235                 240

Val Val Arg Asp Pro Val Thr Arg Ala Ile Ser Asp Tyr Thr Gln Thr
            245                 250                 255

Leu Ser Lys Arg Pro Asp Ile Pro Thr Phe Glu Ser Leu Thr Phe Lys
            260                 265                 270

Asn Arg Thr Ala Gly Leu Ile Asp Thr Ser Trp Ser Ala Ile Gln Ile
            275                 280                 285

Gly Ile Tyr Ala Lys His Leu Glu His Trp Leu Arg His Phe Pro Ile
    290                 295                 300

Arg Gln Met Leu Phe Val Ser Gly Glu Arg Leu Ile Ser Asp Pro Ala
305                 310                 315                 320

Gly Glu Leu Gly Arg Val Gln Asp Phe Leu Gly Leu Lys Arg Ile Ile
            325                 330                 335

Thr Asp Lys His Phe Tyr Phe Asn Lys Thr Lys Gly Phe Pro Cys Leu
            340                 345                 350
```

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Lys Lys Ala Glu Gly Ser Ser Arg Pro His Cys Leu Gly Lys Thr Lys
        355                 360                 365

Gly Arg Thr His Pro Glu Ile Asp Arg Glu Val Val Arg Arg Leu Arg
        370                 375                 380

Glu Phe Tyr Arg Pro Phe Asn Leu Lys Phe Tyr Gln Met Thr Gly His
385                 390                 395                 400

Asp Phe Gly Trp Asp Gly
                405


<210> 9
<211> 2032
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (331)..(1500)
<223> human 3-OST-3B

<400> 9
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agcagaccct cgcccagcag ttaccgccgt cccgactttc cgttccagtt gcagctcctg 120

ccgggcaaca tgtcaagagc cgccgccgct acagctgccg ccgccacctg gggaagagca 180

gcagcagcag cggcggccgc gggcacacgg gggcaataaa ccgagccacc cgggcgtcca 240

gcgtgccggg gaaccctctc tgcgctcact gcccggcggg acccacgcca tgtgctgagc 300

catgtccctg ccgcgccccg cgggcagcgc atg ggg cag cgc ctg agt ggc ggc 354
                                   Met Gly Gln Arg Leu Ser Gly Gly
                                    1                   5

aga tct tgc ctc gat gtc ccc ggc cgg ctc cta ccg cag ccg ccg ccg 402
Arg Ser Cys Leu Asp Val Pro Gly Arg Leu Leu Pro Gln Pro Pro Pro
        10                  15                  20

ccc ccg ccg ccg gtg agg agg aag ctc gcg ctg ctc ttc gcc atg ctc 450
Pro Pro Pro Pro Val Arg Arg Lys Leu Ala Leu Leu Phe Ala Met Leu
25                  30                  35                  40

tgc gtc tgg ctc tat atg ttc ctg tac tcg tgc gcc ggc tcc tgc gcc 498
Cys Val Trp Leu Tyr Met Phe Leu Tyr Ser Cys Ala Gly Ser Cys Ala
                45                  50                  55

gcc gcg ccg ggg ctg ctg ctc ctg ggc tct ggg tcc cgc gcc gca cac 546
Ala Ala Pro Gly Leu Leu Leu Leu Gly Ser Gly Ser Arg Ala Ala His
                60                  65                  70

gac ccg cca gcc ctg gcc aca gct ccg gac ggg acg ccc ccc agg ctg 594
Asp Pro Pro Ala Leu Ala Thr Ala Pro Asp Gly Thr Pro Pro Arg Leu
        75                  80                  85

ccg ttc cgg gcg ccg cca gcc acc cca ctg gct tca ggc aag gag atg 642
Pro Phe Arg Ala Pro Pro Ala Thr Pro Leu Ala Ser Gly Lys Glu Met
        90                  95                  100

gcc gag ggc gct gcg agc ccg gag gag cag agt ccc gag gtg ccg gac 690
Ala Glu Gly Ala Ala Ser Pro Glu Glu Gln Ser Pro Glu Val Pro Asp
105                 110                 115                 120

tcc cca agc ccc atc tcc agc ttt ttc agt ggg tct ggg agc aag cag 738
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Ser Pro Ser Pro Ile Ser Ser Phe Phe Ser Gly Ser Gly Ser Lys Gln
            125   130   135

ctg ccg cag gcc atc atc atc ggc gtg aag aag ggc ggc acg cgg gcg   786
Leu Pro Gln Ala Ile Ile Ile Gly Val Lys Lys Gly Gly Thr Arg Ala
            140   145   150

ctg ctg gag ttt ctg cgc gtg cac ccc gac gtg cgc gcc gtg ggc gcc   834
Leu Leu Glu Phe Leu Arg Val His Pro Asp Val Arg Ala Val Gly Ala
            155   160   165

gag ccc cat ttc ttc gat cgc agc tac gac aag ggc ctc gct tgg tac   882
Glu Pro His Phe Phe Asp Arg Ser Tyr Asp Lys Gly Leu Ala Trp Tyr
            170   175   180

cgg gac ctg atg ccc aga acc ctg gac ggg cag atc acc atg gag aag   930
Arg Asp Leu Met Pro Arg Thr Leu Asp Gly Gln Ile Thr Met Glu Lys
185   190   195   200

acg ccc agt tac ttc gtc acg cgg gag gcc ccc gcg cgc atc tcg gcc   978
Thr Pro Ser Tyr Phe Val Thr Arg Glu Ala Pro Ala Arg Ile Ser Ala
            205   210   215

atg tcc aag gac acc aag ctc atc gtg gtg gtg cgg gac ccg gtg acc   1026
Met Ser Lys Asp Thr Lys Leu Ile Val Val Val Arg Asp Pro Val Thr
            220   225   230

agg gcc atc tcg gac tac acg cag acg ctg tcc aag cgg ccc gac atc   1074
Arg Ala Ile Ser Asp Tyr Thr Gln Thr Leu Ser Lys Arg Pro Asp Ile
            235   240   245

ccc acc ttc gag agc ttg acg ttc aaa aac agg aca gcg ggc ctc atc   1122
Pro Thr Phe Glu Ser Leu Thr Phe Lys Asn Arg Thr Ala Gly Leu Ile
            250   255   260

gac acg tcg tgg agc gcc atc cag atc ggc atc tac gcc aag cac ctg   1170
Asp Thr Ser Trp Ser Ala Ile Gln Ile Gly Ile Tyr Ala Lys His Leu
265   270   275   280

gag cac tgg ctg cgc cac ttc ccc atc cgc cag atg ctc ttc gtg agc   1218
Glu His Trp Leu Arg His Phe Pro Ile Arg Gln Met Leu Phe Val Ser
            285   290   295

ggc gag cgg ctc atc agc gac ccg gcc ggg gag ctg ggc cgc gtg caa   1266
Gly Glu Arg Leu Ile Ser Asp Pro Ala Gly Glu Leu Gly Arg Val Gln
            300   305   310

gac ttc ctg ggc ctc aag agg atc atc acg gac aag cac ttc tac ttc   1314
Asp Phe Leu Gly Leu Lys Arg Ile Ile Thr Asp Lys His Phe Tyr Phe
            315   320   325

aac aag acc aag ggc ttc ccc tgc ctg aag aag gcg gag ggc agc agc   1362
Asn Lys Thr Lys Gly Phe Pro Cys Leu Lys Lys Ala Glu Gly Ser Ser
            330   335   340

cgg ccc cat tgc ctg ggc aag acc aag ggc agg acc cat cct gag atc   1410
Arg Pro His Cys Leu Gly Lys Thr Lys Gly Arg Thr His Pro Glu Ile
345   350   355   360

gac cgc gag gtg gtg cgc agg ctg cgc gag ttc tac cgg cct ttc aac   1458
Asp Arg Glu Val Val Arg Arg Leu Arg Glu Phe Tyr Arg Pro Phe Asn
            365   370   375

ctc aag ttc tac cag atg acc ggg cac gac ttt ggc tgg gat   1500
Leu Lys Phe Tyr Gln Met Thr Gly His Asp Phe Gly Trp Asp
            380   385   390

tgagcagacc cgggctatgt accttaccca cgtggcttat ctattgacag agattatatg 1560

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tatgtaaaat gtacagaaat ctattttata ataatttatt tttaattcat aagcaattaa 1620

ttcactaagc tgcctagcca cactctttag agagttagct tcataatctg ttaacattcc 1680

aaagtgttta actctagtat ttcgttttct tcttcacaat tgatggtgct tctatttttt 1740

cttctcccct acctgttata tttaaaacaa agaaaagcac aacttgagat ttttgttgtt 1800

acgggtattc agccttcagt caccgtctga gttctccagt tgctgcctcc ttgtcttgtc 1860

ttgggtctcc cattccagct tccctgtctc ttcctgcctg tgtacctcgt aggaacgctg 1920

agctgcctca acagggctgt attctgaagg gcaggcctca tgcagcagcc tccttgcaga 1980

tgtggtgtcc cgtccaatga tgtagcctga aagccacagc cctagggttc tg          2032
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<210> 10
<211> 390
<212> PRT
<213> Homo sapiens

<400> 10
Met Gly Gln Arg Leu Ser Gly Gly Arg Ser Cys Leu Asp Val Pro Gly
1               5                   10                  15

Arg Leu Leu Pro Gln Pro Pro Pro Pro Pro Pro Val Arg Arg Lys
            20                  25                  30

Leu Ala Leu Leu Phe Ala Met Leu Cys Val Trp Leu Tyr Met Phe Leu
        35                  40                  45

Tyr Ser Cys Ala Gly Ser Cys Ala Ala Ala Pro Gly Leu Leu Leu Leu
    50                  55                  60

Gly Ser Gly Ser Arg Ala Ala His Asp Pro Pro Ala Leu Ala Thr Ala
65                  70                  75                  80

Pro Asp Gly Thr Pro Pro Arg Leu Pro Phe Arg Ala Pro Pro Ala Thr
                85                  90                  95

Pro Leu Ala Ser Gly Lys Glu Met Ala Glu Gly Ala Ala Ser Pro Glu
            100                 105                 110

Glu Gln Ser Pro Glu Val Pro Asp Ser Pro Ser Pro Ile Ser Ser Phe
        115                 120                 125

Phe Ser Gly Ser Gly Ser Lys Gln Leu Pro Gln Ala Ile Ile Ile Gly
    130                 135                 140

Val Lys Lys Gly Gly Thr Arg Ala Leu Leu Glu Phe Leu Arg Val His
145                 150                 155                 160

Pro Asp Val Arg Ala Val Gly Ala Glu Pro His Phe Phe Asp Arg Ser
                165                 170                 175

Tyr Asp Lys Gly Leu Ala Trp Tyr Arg Asp Leu Met Pro Arg Thr Leu
            180                 185                 190

Asp Gly Gln Ile Thr Met Glu Lys Thr Pro Ser Tyr Phe Val Thr Arg
            195                 200                 205

Glu Ala Pro Ala Arg Ile Ser Ala Met Ser Lys Asp Thr Lys Leu Ile
        210                 215                 220

Val Val Val Arg Asp Pro Val Thr Arg Ala Ile Ser Asp Tyr Thr Gln
225                 230                 235                 240
```

Thr Leu Ser Lys Arg Pro Asp Ile Pro Thr Phe Glu Ser Leu Thr Phe
              245   250   255

Lys Asn Arg Thr Ala Gly Leu Ile Asp Thr Ser Trp Ser Ala Ile Gln
              260   265   270

Ile Gly Ile Tyr Ala Lys His Leu Glu His Trp Leu Arg His Phe Pro
          275   280   285

Ile Arg Gln Met Leu Phe Val Ser Gly Glu Arg Leu Ile Ser Asp Pro
      290   295   300

Ala Gly Glu Leu Gly Arg Val Gln Asp Phe Leu Gly Leu Lys Arg Ile
305   310   315   320

Ile Thr Asp Lys His Phe Tyr Phe Asn Lys Thr Lys Gly Phe Pro Cys
              325   330   335

Leu Lys Lys Ala Glu Gly Ser Ser Arg Pro His Cys Leu Gly Lys Thr
              340   345   350

Lys Gly Arg Thr His Pro Glu Ile Asp Arg Glu Val Val Arg Arg Leu
          355   360   365

Arg Glu Phe Tyr Arg Pro Phe Asn Leu Lys Phe Tyr Gln Met Thr Gly
      370   375   380

His Asp Phe Gly Trp Asp
385   390

<210> 11
<211> 3658
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (847)..(2214)

<220>
<223> Predicted human 3-OST-4 hnRNA

<400> 11
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aaaagcggaa gcgagagctt cgtcacccgc tgtcttccag ctcccggtgc gcggcaccgg 120

aggcaggcgt tgggctttac ctctctaaaa gtactggggc aaaggaatgg agaacacggc 180

gtcccgagct cccaagggag gggagtaaac gaggtggggt ggggaacacc ccaagtgcgt 240

gcgtgctggg gggctggggg gcacgatctc cgttctcccg ggtgccccag ccctagcgca 300

cgcctccgct cccccgcccc cttcgcaggc gcgcgcgagg cgcacccccc ttccctcggc 360

ggcgccgggc gcgcgcccgg cccctcctc ctcccctccg cgcctctcct ctctcccggc 420

agaaagttag cagcggggaa ggaactctgg gctgcaacag cgcgcggcgg cggcggcaga 480

ggctgaagca gaagccgcgg cggagccggg gaagcggggg cgctgcagac ggagcaggtg 540

ccgccggcgg gtccgcgcgc cccctcggt ccccttgcct gaggctgagg ggggggcggt 600

ggtggggggg ccactcggac tcggcgggca gcgtggggcg gggggccatg cggccgggct 660

cccccctggc gcagcgggac agcggccagg gccggggggcg cagcggcgtc gcttcatgca 720

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gccggggcgg ctgggcagcg gcggcggcgg cggcggcggc ggcggcgggg gcggcggctg 780

aaaccatgtc cgggcagcgc cggggctgc cgccgccgcc gccgccgccg cgagccggga 840

gccgcg atg gcc cgg tgg ccc gca cct cct ccg cct ccg cct ccg cct  888
       Met Ala Arg Trp Pro Ala Pro Pro Pro Pro Pro Pro Pro Pro
       1               5                   10

cca cct ctg gcc gcg ccg ccg ccg ccc ggc gcc tct gct aag ggg ccg  936
Pro Pro Leu Ala Ala Pro Pro Pro Pro Gly Ala Ser Ala Lys Gly Pro
15              20              25                  30

ccg gcg cgc aag ctg ctt ttt atg tgc acc ttg tcc ctg tct gtc acc  984
Pro Ala Arg Lys Leu Leu Phe Met Cys Thr Leu Ser Leu Ser Val Thr
            35                  40                  45

tac ctg tgc tac agc ctc ctg ggc ggc tcg ggc tcc ctg caa ttc cct  1032
Tyr Leu Cys Tyr Ser Leu Leu Gly Gly Ser Gly Ser Leu Gln Phe Pro
            50                  55                  60

ctg gcg ctg cag gag tcg ccg ggc gcc gcc gcc gag ccc ccg ccg agc  1080
Leu Ala Leu Gln Glu Ser Pro Gly Ala Ala Ala Glu Pro Pro Pro Ser
        65                  70                  75

ccg ccg cca ccc tct ctg ctg cct acc ccc gtg cgc ctc ggc gcc ccc  1128
Pro Pro Pro Pro Ser Leu Leu Pro Thr Pro Val Arg Leu Gly Ala Pro
        80                  85                  90

tcg cag ccg ccc gcg ccg ccg ccg ctg gac aac gcg agc cac ggg gag  1176
Ser Gln Pro Pro Ala Pro Pro Pro Leu Asp Asn Ala Ser His Gly Glu
95                  100                 105                 110

ccg ccc gag ccc cca gag cag cca gcc gcc ccc ggg acc gac ggc tgg  1224
Pro Pro Glu Pro Pro Glu Gln Pro Ala Ala Pro Gly Thr Asp Gly Trp
                115                 120                 125

ggg ctg ccg agc ggc ggc gga ggc gcc cgg gac gcc tgg ctc cgg acc  1272
Gly Leu Pro Ser Gly Gly Gly Gly Ala Arg Asp Ala Trp Leu Arg Thr
            130                 135                 140

ccg ctg gcc ccc agc gag atg atc acg gct cag agc gcg ctg ccg gag  1320
Pro Leu Ala Pro Ser Glu Met Ile Thr Ala Gln Ser Ala Leu Pro Glu
        145                 150                 155

agg gaa gcg cag gag tcc agc acc acc gac gag gat ctc gca ggc cgg  1368
Arg Glu Ala Gln Glu Ser Ser Thr Thr Asp Glu Asp Leu Ala Gly Arg
    160                 165                 170

aga gcg gcc aac ggg agc agc gag agg ggc ggc gcc gtc agc acc ccc  1416
Arg Ala Ala Asn Gly Ser Ser Glu Arg Gly Gly Ala Val Ser Thr Pro
175                 180                 185                 190

gac tat ggg gag aag aag ctg cca cag gcg ctc atc atc ggg gtc aag  1464
Asp Tyr Gly Glu Lys Lys Leu Pro Gln Ala Leu Ile Ile Gly Val Lys
                195                 200                 205

aaa gga ggg acc cgc gcg ctg ctg gag gcg atc cgc gtg cac ccg gac  1512
Lys Gly Gly Thr Arg Ala Leu Leu Glu Ala Ile Arg Val His Pro Asp
            210                 215                 220

gtg cgg gcg gtg ggc gta gag ccg cac ttc ttc gac agg aac tac gaa  1560
Val Arg Ala Val Gly Val Glu Pro His Phe Phe Asp Arg Asn Tyr Glu
        225                 230                 235

aag ggg ttg gag tgg tac aga aat gtg atg ccc aag act ttg gat ggg  1608
Lys Gly Leu Glu Trp Tyr Arg Asn Val Met Pro Lys Thr Leu Asp Gly
    240                 245                 250
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caa ata acc atg gag aag act cca agt tac ttt gtg aca aat gag gct    1656
Gln Ile Thr Met Glu Lys Thr Pro Ser Tyr Phe Val Thr Asn Glu Ala
255             260             265             270

ccc aag cgc att cac tcc atg gcc aag gac atc aaa ctg att gtg gtg    1704
Pro Lys Arg Ile His Ser Met Ala Lys Asp Ile Lys Leu Ile Val Val
            275             280             285

gtg aga aac ccc gtg acc agg gcc atc tct gac tac acg cag aca ctg    1752
Val Arg Asn Pro Val Thr Arg Ala Ile Ser Asp Tyr Thr Gln Thr Leu
            290             295             300

tca aag aaa ccc gag atc ccc acc ttt gag gtg ctg gcc ttc aaa aac    1800
Ser Lys Lys Pro Glu Ile Pro Thr Phe Glu Val Leu Ala Phe Lys Asn
            305             310             315

cgg acc ctc ggg ctg atc gat gct tcc tgg agt gcc att cga ata ggg    1848
Arg Thr Leu Gly Leu Ile Asp Ala Ser Trp Ser Ala Ile Arg Ile Gly
    320             325             330

atc tat gcg ctg cat ctg gaa aac tgg ctc cag tat ttc ccc ctc tcc    1896
Ile Tyr Ala Leu His Leu Glu Asn Trp Leu Gln Tyr Phe Pro Leu Ser
335             340             345             350

cag atc ctc ttt gtc agt ggt gag cga ctc att gtg gac ccc gcc ggg    1944
Gln Ile Leu Phe Val Ser Gly Glu Arg Leu Ile Val Asp Pro Ala Gly
            355             360             365

gaa atg gcc aaa gta cag gat ttt cta ggc ctc aaa cgt gtt gtg act    1992
Glu Met Ala Lys Val Gln Asp Phe Leu Gly Leu Lys Arg Val Val Thr
            370             375             380

aag aag cat ttc tat ttc aac aaa acc aag ggg ttc cct tgc cta aag    2040
Lys Lys His Phe Tyr Phe Asn Lys Thr Lys Gly Phe Pro Cys Leu Lys
            385             390             395

aag cca gaa gac agc agt gcc ccg agg tgc tta ggc aag agc aaa ggt    2088
Lys Pro Glu Asp Ser Ser Ala Pro Arg Cys Leu Gly Lys Ser Lys Gly
    400             405             410

cgg act cat cct cgc att gac cca gat gtc atc cac aga ctg agg aaa    2136
Arg Thr His Pro Arg Ile Asp Pro Asp Val Ile His Arg Leu Arg Lys
415             420             425             430

ttc tac aaa ccc ttc aac ttg atg ttt tac caa atg act ggt caa gat    2184
Phe Tyr Lys Pro Phe Asn Leu Met Phe Tyr Gln Met Thr Gly Gln Asp
            435             440             445

ttt cag tgg gaa cag gaa gag ggt gat aaa tgaggctaga gaggcagagg     2234
Phe Gln Trp Glu Gln Glu Glu Gly Asp Lys
            450             455

aaggctagtc aataagctaa ggaggctcct tgcctgagtc cttgaatacc ccagcttctg   2294

cagcttcact tgctggagtg ccaagtagat ctcctcctcc ttcatgcagc caggattgcc   2354

tccagtgctg ttagcttagg caaacaggtg gatcccatgg catccccatg gaggaaccag   2414

gcccatctgg gcagcagcat ctggttgacc agatggccac cagaacccac tgttcattct   2474

tatcttctgc tagttaatat agcctgaaga cagaggataa atagttgtca atgtcagaga   2534

cagtgctatt aatgtatatg tgagcgacaa aaaaggtctg ctttataggg gttctcactc   2594

tagcttgggg agcccagggt tctagccctg tatctgtcat gggcacctgc tgtctaaacc   2654

tctgcttggg cttctcccca gaatgcactt tgtggctgag tgctccagga ctcctaggga   2714
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gcaagctcct ccctctaagg tgtttctagt cttctcttta aaggtctcat cccacaaccc 2774

ctgacttcct ccctccccac atcatgaagg cagaggcatg cacattcctc actgaaaaag 2834

aaaacacaca cccacccaca cacacacaca cagaagaaaa tgaaagctga cacacctcga 2894

agccttcttt ccaagagccc tctaaatggg gttgggtctc actcttcatg agtatcctgg 2954

gttgtgcaga agcttagcat atgcccttgt gttcggatca ggcccacagg gctgctcaaa 3014

gagtagagta attgtaaccg aggtcagagc tctggggttg gcagagatga gtggccatat 3074

ctgggggtaa aagaagaaat cctgtcctct tggtgggagg ttaccttacc tgaagaccat 3134

ctctcccaag cactgtagtt ctgagcatgt ttttggggtg gactctgtcc cctagggtcc 3194

ctagaagggc aaagaccaga gagttgacaa gtctgttatt aggaataatc cttagccatg 3254

taatggagaa aggagcagtc agcattcttc caatttgccc caccaccacc tcctcgggct 3314

tcattttctc tatttagaga tggcagagag tgaggtagtg gcgagaaagc tgactccatt 3374

catcagatcc agtttatgag ggttgggggt gagcaagggc tgtctgcaga aaccccatc 3434

aagagctgct gaatgaagtg tcccttccca tcagtttgat tcaattaaaa tgcatcattt 3494

gacataaagc acttgttcac agatctccaa aaccaggaat tgttctagta aaactggaaa 3554

tttgtatgag tggggggagt taaatctgtt cagctgttat taaactgtca tttctcccgc 3614

taaatgaaaa ccgtgttgtt ataaagctta atgcaacctg atta                3658
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<210> 12
<211> 456
<212> PRT
<213> Homo sapiens

<400> 12
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Met Ala Arg Trp Pro Ala Pro Pro Pro Pro Pro Pro Pro Pro Pro
 1               5                  10                  15

Leu Ala Ala Pro Pro Pro Pro Gly Ala Ser Ala Lys Gly Pro Pro Ala
            20                  25                  30

Arg Lys Leu Leu Phe Met Cys Thr Leu Ser Leu Ser Val Thr Tyr Leu
        35                  40                  45

Cys Tyr Ser Leu Leu Gly Gly Ser Gly Ser Leu Gln Phe Pro Leu Ala
    50                  55                  60

Leu Gln Glu Ser Pro Gly Ala Ala Ala Glu Pro Pro Ser Pro Pro
65                  70                  75                  80

Pro Pro Ser Leu Leu Pro Thr Pro Val Arg Leu Gly Ala Pro Ser Gln
            85                  90                  95

Pro Pro Ala Pro Pro Pro Leu Asp Asn Ala Ser His Gly Glu Pro Pro
            100                 105                 110

Glu Pro Pro Glu Gln Pro Ala Ala Pro Gly Thr Asp Gly Trp Gly Leu
        115                 120                 125

Pro Ser Gly Gly Gly Gly Ala Arg Asp Ala Trp Leu Arg Thr Pro Leu
    130                 135                 140

Ala Pro Ser Glu Met Ile Thr Ala Gln Ser Ala Leu Pro Glu Arg Glu
```

145   150   155   160

Ala Gln Glu Ser Ser Thr Thr Asp Glu Asp Leu Ala Gly Arg Arg Ala
            165   170   175

Ala Asn Gly Ser Ser Glu Arg Gly Gly Ala Val Ser Thr Pro Asp Tyr
            180   185   190

Gly Glu Lys Lys Leu Pro Gln Ala Leu Ile Ile Gly Val Lys Lys Gly
            195   200   205

Gly Thr Arg Ala Leu Leu Glu Ala Ile Arg Val His Pro Asp Val Arg
    210   215   220

Ala Val Gly Val Glu Pro His Phe Phe Asp Arg Asn Tyr Glu Lys Gly
225   230   235   240

Leu Glu Trp Tyr Arg Asn Val Met Pro Lys Thr Leu Asp Gly Gln Ile
            245   250   255

Thr Met Glu Lys Thr Pro Ser Tyr Phe Val Thr Asn Glu Ala Pro Lys
            260   265   270

Arg Ile His Ser Met Ala Lys Asp Ile Lys Leu Ile Val Val Val Arg
            275   280   285

Asn Pro Val Thr Arg Ala Ile Ser Asp Tyr Thr Gln Thr Leu Ser Lys
    290   295   300

Lys Pro Glu Ile Pro Thr Phe Glu Val Leu Ala Phe Lys Asn Arg Thr
305   310   315   320

Leu Gly Leu Ile Asp Ala Ser Trp Ser Ala Ile Arg Ile Gly Ile Tyr
            325   330   335

Ala Leu His Leu Glu Asn Trp Leu Gln Tyr Phe Pro Leu Ser Gln Ile
            340   345   350

Leu Phe Val Ser Gly Glu Arg Leu Ile Val Asp Pro Ala Gly Glu Met
            355   360   365

Ala Lys Val Gln Asp Phe Leu Gly Leu Lys Arg Val Val Thr Lys Lys
    370   375   380

His Phe Tyr Phe Asn Lys Thr Lys Gly Phe Pro Cys Leu Lys Lys Pro
385   390   395   400

Glu Asp Ser Ser Ala Pro Arg Cys Leu Gly Lys Ser Lys Gly Arg Thr
            405   410   415

His Pro Arg Ile Asp Pro Asp Val Ile His Arg Leu Arg Lys Phe Tyr
            420   425   430

Lys Pro Phe Asn Leu Met Phe Tyr Gln Met Thr Gly Gln Asp Phe Gln
            435   440   445

Trp Glu Gln Glu Glu Gly Asp Lys
    450   455

<210> 13
<211> 284
<212> PRT
<213> Homo sapiens

<220>
<223> human NST-1 (aa 599 to 882)

<400> 13
Lys Thr Cys Asp Arg Phe Pro Lys Leu Leu Ile Ile Gly Pro Gln Lys
1   5   10   15

Thr Gly Thr Thr Ala Leu Tyr Leu Phe Leu Gly Met His Pro Asp Leu
            20   25   30

Ser Ser Asn Tyr Pro Ser Ser Glu Thr Phe Glu Glu Ile Gln Phe Phe
        35   40   45

Asn Gly His Asn Tyr His Lys Gly Ile Asp Trp Tyr Met Glu Phe Phe
    50   55   60

Pro Ile Pro Ser Asn Thr Thr Ser Asp Phe Tyr Phe Glu Lys Ser Ala
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Asn Tyr Phe Asp Ser Glu Val Ala Pro Arg Arg Ala Ala Ala Leu Leu
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Pro Lys Ala Lys Val Leu Thr Ile Leu Ile Asn Pro Ala Asp Arg Ala
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Tyr Ser Trp Tyr Gln His Gln Arg Ala His Asp Asp Pro Val Ala Leu
        115   120   125

Lys Tyr Thr Phe His Glu Val Ile Thr Ala Gly Ser Asp Ala Ser Ser
    130   135   140

Lys Leu Arg Ala Leu Gln Asn Arg Cys Leu Val Pro Gly Trp Tyr Ala
145   150   155   160

Thr His Ile Glu Arg Trp Leu Ser Ala Tyr His Ala Asn Gln Ile Leu
            165   170   175

Val Leu Asp Gly Lys Leu Leu Arg Thr Glu Pro Ala Lys Val Met Asp
            180   185   190

Met Val Gln Lys Phe Leu Gly Val Thr Asn Thr Ile Asp Tyr His Lys
        195   200   205

Thr Leu Ala Phe Asp Pro Lys Lys Gly Phe Trp Cys Gln Leu Leu Glu
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Gly Gly Lys Thr Lys Cys Leu Gly Lys Ser Lys Gly Arg Lys Tyr Pro
225   230   235   240

Glu Met Asp Leu Asp Ser Arg Ala Phe Leu Lys Asp Tyr Tyr Arg Asp
            245   250   255

His Asn Ile Glu Leu Ser Lys Leu Leu Tyr Lys Met Gly Gln Thr Leu
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Pro Thr Trp Leu Arg Glu Asp Leu Gln Asn Thr Arg
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<220>
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Thr Ser Ser Phe Pro Ser Pro Ser Thr Phe Glu Glu Ile Gln Phe Phe
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Asn Ser Pro Asn Tyr His Lys Gly Ile Asp Trp Tyr Met Asp Phe Phe
        50   55   60

Pro Val Pro Ser Asn Ala Ser Thr Asp Phe Leu Phe Glu Lys Ser Ala
65   70   75   80

Thr Tyr Phe Asp Ser Glu Val Val Pro Arg Arg Gly Ala Ala Leu Leu
                85   90   95

Pro Arg Ala Lys Ile Ile Thr Val Leu Thr Asn Pro Ala Asp Arg Ala
            100   105   110

Tyr Ser Trp Tyr Gln His Gln Arg Ala His Gly Asp Pro Val Ala Leu
        115   120   125

Asn Tyr Thr Phe Tyr Gln Val Ile Ser Ala Ser Ser Gln Thr Pro Leu
    130   135   140

Ala Leu Arg Ser Leu Gln Asn Arg Cys Leu Val Pro Gly Tyr Tyr Ser
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Thr His Leu Gln Arg Trp Leu Thr Tyr Tyr Pro Ser Gly Gln Leu Leu
            165   170   175

Ile Val Asp Gly Gln Glu Leu Arg Thr Asn Pro Ala Ala Ser Met Glu
            180   185   190

Ser Ile Gln Lys Phe Leu Gly Ile Thr Pro Phe Leu Asn Tyr Thr Arg
        195   200   205

Thr Leu Arg Phe Asp Asp Asp Lys Gly Phe Trp Cys Gln Gly Leu Glu
    210   215   220

Gly Gly Lys Thr Arg Cys Leu Gly Arg Ser Lys Gly Arg Arg Tyr Pro
225   230   235   240

Asp Met Asp Thr Glu Ser Arg Leu Phe Leu Thr Asp Phe Phe Arg Asn
            245   250   255

His Asn Leu Glu Leu Ser Lys Leu Leu Ser Arg Leu Gly Gln Pro Val
            260   265   270

Pro Ser Trp Leu Arg Glu Glu Leu Gln His Ser Ser Leu Gly
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Cys Gly Val Ile Pro Asn Thr Ser Lys Lys Arg Phe Pro Asp Ala Ile
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Ile Val Gly Val Lys Lys Ser Gly Thr Arg Ala Leu Leu Glu Phe Leu

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    Arg Val Asn Pro Leu Ile Lys Ala Pro Gly Pro Glu Val His Phe Phe
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    Asp Lys Asn Phe Asn Lys Gly Leu Glu Trp Tyr Arg Glu Gln Met Pro
     65                    70                    75                    80

    Glu Thr Lys Phe Gly Glu Val Thr Ile Glu Lys Ser Pro Ala Tyr Phe
                      85                    90                    95

    His Ser Lys Met Ala Pro Glu Arg Ile Lys Ser Leu Asn Pro Asn Thr
                      100                   105                   110

    Lys Ile Ile Ile Val Val Arg Asp Pro Val Thr Arg Ala Ile Ser Asp
                      115                   120                   125

    Tyr Thr Gln Ser Ser Ser Lys Arg Lys Arg Val Gly Leu Met Pro Ser
         130                   135                   140

    Phe Glu Thr Met Ala Val Gly Asn Cys Ala Asn Trp Leu Arg Thr Asn
    145                   150                   155                   160

    Cys Thr Thr Lys Thr Arg Gly Val Asn Ala Gly Trp Gly Ala Ile Arg
                      165                   170                   175

    Ile Gly Val Tyr His Lys His Met Lys Arg Trp Leu Asp His Phe Pro
                      180                   185                   190

    Ile Glu Asn Ile His Ile Val Asp Gly Glu Lys Leu Ile Ser Asn Pro
                      195                   200                   205

    Ala Asp Glu Ile Ser Ala Thr Glu Lys Phe Leu Gly Leu Lys Pro Val
         210                   215                   220

    Ala Lys Pro Glu Lys Phe Gly Val Asp Pro Ile Lys Lys Phe Pro Cys
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    Ile Lys Asn Glu Asp Gly Lys Leu His Cys Leu Gly Lys Thr Lys Gly
                      245                   250                   255

    Arg His His Pro Asp Val Glu Pro Ser Val Leu Lys Thr Leu Arg Glu
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    Phe Tyr Gly Pro Glu Asn Lys Lys Phe Tyr Gln Met Ile Asn His Trp
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    Phe Asp Trp
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